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CYTOLOGICAL CHANGES INDUCED IN THE HYPOPHYSIS BY THE PROLONGED ADMINISTRATION OF PITUITARY EXTRACT *

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When Harvey Cushing first described the syndrome that now bears his name he promptly appreciated the fact that pituitary basophilism, as was true of acromegaly (*i.e.* pituitary acidophilism), would be better understood if it could be experimentally reproduced. An attempt to achieve this result by the injection into a puppy of a crude pituitary gonadotropic extract (presumably of basophilic elements) was described in 1934 by Thompson and Cushing.[30] While the animal developed a condition bearing certain resemblances to the clinical syndrome, it showed at the same time effects more or less characteristic of pituitary deficiency, namely, inactivation of the thyroid, adrenal cortex and gonads, and a failure to grow.

Equally striking results obtained in attempts to reproduce the syndrome in other animals subsequently led one of us (K. W. T.) to make a correlated study of the antihormones, discovered by Collip and his co-workers,[1, 3, 4] in order to interpret the rôles these substances may have played in the injected animals. These

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studies established the non-species-specific quality of the antihormones, [15, 27, 31] and accounted for the observed inactivation of the animals' own pituitary hormones.

The evidence now available from several sources (Du Shane *et al.*,[6] Gordon *et al.*,[7] Rowlands and Parkes,[15, 16] and Twombly [32]) suggests that the antihormones are antibodies which are formed in the injected animal in response to an antigen to which is linked the pituitary hormone. The antihormones are readily produced when crude pituitary extracts from a foreign species are injected into an animal, but not when these extracts are sufficiently purified or the antigenic complex is separated from the hormone (Werner,[35, 36, 37] confirmed by Thompson, unpublished experiments). These antisubstances have not usually developed when the test animal has been injected with extracts derived from the same species (sheep pituitaries in sheep, Rowlands,[14] Thompson,[26] Collip [3*]; rat pituitaries in rats, Smith [24, 25]; and prolan in man, Twombly [32]). When first developed in the animal, the antihormone is ordinarily specific only for the extract injected, but after prolonged injections under suitable conditions the antihormones become non-species-specific; in other words, they inactivate the like hormones from many species of animals including those of the injected animal itself (unpublished data, Thompson).

Definite physiological effects are caused by the antihormones in animals. The gonadotropic antihormone, for example, under suitable conditions causes: (1) an inactivation in test rats of gonadotropic hormones from several species of animals, including man [15, 30]; (2) the failure of the sexual maturity of young rats [30]; and (3) the abortion of mice,[30] rats,[30] rabbits [11, 30] and dogs [29] (the latter 2 species may thus be aborted at any stage of pregnancy). Anderson and Collip have reported the lowering of the basal metabolic rate of animals treated with thyrotropic antihormone.[1, 4]

While these antihormone studies were in progress, clinical case reports of the so-called Cushing syndrome from various sources made it clear that a condition almost indistinguishable from that accompanying a basophilic adenoma may occur with an adenoma or carcinoma of the adrenal cortex,[2, 8] an oat-cell tumor or carci-

* Collip observed weak antihormones in the serum of 2 out of 4 sheep that had been injected with sheep extract. It is conceivable that denaturation of the extract may have been responsible for the results.

noma of the thymus,[9] and in a few instances with no demonstrable adenoma of any organ (unreported cases). Furthermore, Crooke [5] and later Rasmussen [12] noted a characteristic cytoplasmic hyalinization in the basophilic cells of the pituitary in verified cases with the syndrome. This change is found in most of the basophilic cells of the anterior lobe, but it does not occur in the cells of the basophilic adenoma itself, should one be present. These specific changes have been observed only in the glands removed from human cases and heretofore have not been described in experimental animals. Crooke claimed this basophilic hyalinization to be the only single "pathological common denominator," possibly the essential lesion, of the syndrome.

An analysis of the data concerned with the Cushing syndrome made it appear more than likely that in the antihormone experiments some associated changes in the anterior pituitary glands of the animals might also be expected, and certainly should be sought.

The present report concerns itself with a study of the cytological changes in the pituitary glands of three groups of animals (A, B and C), some of which had been subjects for the attempts to reproduce the clinical syndrome of basophilism, and others of which were subjects for the investigation of the antihormones.

The pituitaries first studied were those from 2 dogs (A) that had received prolonged injections of a crude sheep pituitary extract. Marked cytological changes appeared in these glands. It was impossible to say whether the changes in these two hypophyses were due directly to the injected extract or to the physiological action of the antihormones which were extremely active in the serums of these animals. There are, to the authors' knowledge, no cytological data to indicate what effect, if any, the antihormones have upon the anterior pituitary gland. To this end 2 other dogs (B) were injected with a suitable antihormone serum, in order that their hypophyses might be studied. The third pair of glands to be studied were the hypophyses of 2 sheep (C) which did not develop antihormones during a prolonged course of injections of the sheep extract. Before proceeding to a cytological description of the hypophyses of these animals, it seems advisable to state briefly the experimental observations.

The Animals

(*A*) *The Dogs Injected with Sheep Pituitary Extract*

Dog No. 1: A female fox terrier puppy was injected daily for 4 months with 25 cc. of an extract of sheep pituitary glands. This extract, which had been used in many of the antihormone experiments of one of the authors (K. W. T.), was prepared by alcohol precipitation after a method described by van Dyke and Wallen-Lawrence.[33] A marked atrophy of the thyroid, adrenals and gonads was noted and reported in the aforementioned publication (Thompson and Cushing[30]). The grossly normal pituitary gland was preserved for future study. The antihormones were not investigated in this animal, but later observations of other puppies similarly treated have indicated that the effects observed may be attributed largely to these very active antisubstances.

Dog No. 2: An adult female shepherd dog was injected daily for 210 days with 25 cc. of an extract identical with that given to dog No. 1. The animal at first developed in her serum the augmentary principle (Thompson[28]), and she later developed, in succession, antihormones for the gonadotropic hormone of sheep pituitary extract, pregnant mare serum, and human pituitary glands. During the period of the injections this animal's fur became coarse and sparse, as if she had been thyroidectomized, and she developed hypercholesterolemia. At autopsy the thyroid, adrenals and gonads were found to be atrophied. The thyroid epithelium, fixed in Zenker's plus acetic acid, was flattened, and the colloid had no absorption vacuoles.

(*B*) *The Dogs Injected with Canine Antihormone*

Dog No. 3: An adult female mongrel dog was injected subcutaneously daily for a period of 32 days with 10 cc. of canine antihormone serum. The donor was a Collie dog that had been injected daily for 3 years with the above mentioned sheep pituitary extract. This particular serum inactivated the gonadotropic hormones of many species, and it invariably produced abortion of pregnant dogs. In addition to the antihormones the serum contained considerable amounts of the antidiuretic principle, which

also was present in the sheep extract. The serum did not contain a measurable amount of the oxytocic principle.*

At autopsy the pituitary gland of the dog injected with this serum was grossly normal, but the thyroid, adrenals and gonads appeared smaller than normal. Histologically the thyroid showed all the signs of subnormal functional activity. This animal did not develop hypercholesterolemia.

Dog No. 4: An adult female mongrel dog was injected daily for 30 days with 10 cc. of the same antiserum. The serum in this case was injected intramuscularly on one day and intravenously the next day because the intravenous route of administration was found to be more satisfactory for the induction of abortion of pregnant dogs. At autopsy the thyroid, adrenals and gonads appeared inactive. Histologically the thyroid was slightly more active than that of dog No. 3, but it was no more active than normal. This animal also failed to show hypercholesterolemia.

(*C*) *The Sheep Injected with Sheep Pituitary Extract*

Ewe No. 1: An immature ewe was injected daily, beginning at the age of 4 months, with 25 cc. of the same sheep pituitary extract that was given to dogs Nos. 1 and 2. During the subsequent 6 months this ewe's serum was tested at intervals and was found to contain no gonadotropic antihormone. Before the injections were started, however, the serum contained a principle which inactivated thyrotropic hormone. During the period of injections the genitalia and nipples continually showed signs of stimulation, and at autopsy, after 6 months of injections, the internal genitalia were found to be considerably hypertrophied. As compared to a half-sister control of the same age, the adrenals and thyroid were approximately normal. Histologically the thyroid showed a normal degree of activity. The hypophysis was normal in size.

Ewe No. 2: This ewe, a twin of ewe No. 1, was subjected to similar treatment except that the extract injected was prepared by alkaline extraction of an acetone-dried powder of sheep pituitary glands. This animal did not develop gonadotropic antihor-

* For the tests of the posterior lobe principles, the authors are indebted to Dr. Alfred Z. Gilman of the Laboratory of Pharmacology, Yale University Medical School, New Haven, Conn.

mones, and the autopsy revealed the same effects of the injections as were found in ewe No. 1.

Ewe No. 3: The control lamb was a half-sister of ewes Nos. 1 and 2. Their parents were from a highly inbred stock. The control was fed approximately the same diet and was 10 months old when autopsied. Her serum contained no gonadotropic antihormone, but, like the other 2 lambs, contained an antithyrotropic principle. Her gonads and uterus were not hypertrophied.

The Cytology of the Pituitary Gland

Technical Methods: The pituitary gland of dog No. 1 was removed 2 hours after death and fixed in neutral 10 per cent formalin. All other hypophyses were removed promptly after the death of the animal by air embolism and immediately fixed in Zenker-formalin.

A brief outline of the technic used to prepare the tissues for microscopic examination is given below:

1. After 8–24 hours fixation, tissues are washed in running water for 15 hours and then run through graded alcohols to 95 per cent alcohol where they remain for 5–12 hours, depending on the size of the tissue block. The tissue is then placed in absolute alcohol to which is added an equal volume of ether. After 2 hours, an equal volume of 2 per cent celloidin is added. The tissue then remains in this mixture for 5–18 hours.

2. Tissue is run through celloidin as follows: 48 hours in 2 per cent, 48 hours in 4 per cent, and 48 hours in 6 per cent. The tissue is then cast in 6 per cent celloidin in paper boxes and is hardened overnight in chloroform vapor in an air-tight jar. Next, trim the celloidin as close to the tissue as possible and place the block in carbon disulphide for 24 hours or longer if the block continues to float.

3. Place the block in carbon disulphide-paraffin mash (equal parts 62° C. paraffin and carbon disulphide) for 5–7 days in a place just sufficiently warm to keep the paraffin melted. Carry the block through 1 change of melted 62° C. paraffin for from 5–25 minutes and cast in freshly filtered 62° C. paraffin which has been heated to about 70° C. Immerse in warm water (not over 45° C.) for hardening.

4. If the tissues are brittle or difficult to cut, shave the block

until a small portion of the tissue is exposed and submerge in water for from 2 to 5 days before sectioning. Ice, or ice water, is usually necessary to keep the knife and the block cold during cutting at 2 or 3 μ.

5. The following mixture is used for mounting and spreading the sections (after egg albumin has been applied to the slide). To 10 cc. of acetone add 5 drops of methylbenzoate and mix well; add 40 cc. of distilled water. In spreading the sections, the best results are obtained by rapid cautious use of a hot plate at a temperature 5–10 degrees higher than the melting point of the paraffin used.

Staining: Sections are run through xylol and absolute alcohol into a solution of 3 parts of oil of cloves and 1 part of absolute alcohol for 10 minutes. Proceed through graded alcohols to distilled water. Flood the slides with Altmann's 20 per cent acid fuchsin solution and gently heat to steaming (with an alcohol lamp). Allow 5 minutes for cooling. Differentiate, if necessary, in picric acid alcohol as recommended in the Altmann method (1 part of saturated alcoholic picric acid and 7 parts of 20 per cent alcohol).

Wash the slides carefully in distilled water and place in 1 per cent phosphomolybdic acid for from 1 to 2 hours. Place the slides directly (do not rinse) into aniline blue as prepared by Masson for about 1 hour (longer or shorter time as required). Wash the slides in distilled water and shake off the excess water. Rinse quickly in 95 per cent alcohol and absolute alcohol, clear in xylol and mount.

If desired, hematoxylin may be successfully used as a nuclear stain. It should precede the acid fuchsin.

(*A*) *Anterior Hypophyses of Dogs Injected for an Extended Period with Sheep Pituitary Extract*

The hypophyses of dogs Nos. 1 and 2 are profoundly altered. Although essentially similar, the cytological changes are more pronounced in dog No. 2 and may be attributed to the much longer period of injection. The basophils are much larger than normal. In some the distinct cytoplasmic granulation, characteristic of the basophils in dogs, is still present. In many cells, however, the character of the granulation has changed. Granules are frequently

gathered into spherical masses of irregular size, as shown in Figures 1–6. While such cells may be found by search in the normal hypophyses, there is no question of their great numerical increase in these injected dogs.

Most of the basophils show extensive vacuolation. The vacuoles are of three types. In the first type they appear as clear spaces, suggesting that they contain a non-stainable substance, or that the original substance has been technically removed (Figs. 19–24). A second group of vacuoles is filled with a substance staining a clear pale blue. These vacuoles in early stages are small and scattered throughout the cytoplasm. In other cells the vacuoles have coalesced to form more extensive vacuolar inclusions. A third type, perhaps the most common, has a deeply basophilic amorphous substance. Here again, many cells will show small vacuoles distributed among the coarse masses of basophilic granules (Figs. 7–17). In later stages the vacuoles have expanded and united to occupy large portions of the cell, or have replaced the granular cytoplasm almost entirely. Figures 11 and 12 show that the large vacuoles are formed by coalescence of the smaller ones.

In this widespread disturbance of the basophils it is readily possible to demonstrate cells in which the vacuolation is identical with that in the typical castration cell of the rat or monkey (Fig. 17). Other basophils contain the more extensive irregular vacuolar distortion regularly seen in the hypophysis of the thyroidectomized rat (Fig. 22). Still other cells show a combination of granular and liquefied areas which are indistinguishable from the hyalinization of basophils described by Crooke in the Cushing syndrome of pituitary basophilism (Fig. 31).

The fact is evident that regardless of their final configurations, the types of vacuolation begin in a similar manner, essentially a liquefaction of the cytoplasmic granules to give a basophilic amorphous substance, perhaps better called colloid-like than hyaline. Previous careful cytological analysis of the onset of basophilic changes which follow castration and thyroidectomy have shown that the early stages of vacuolation are indistinguishable. Experimental evidence supports the contention that these vacuolations are inseparable for it is possible to prevent or remove vacuolation after thyroidectomy by administration of estrone in doses still within the physiological range (2–5 r. u. per day)

(Severinghaus,[22] and Nelson and Hickman [10]). Castration changes, conversely, have not been cleared up by the administration of thyroxin. One might expect that this would be difficult to accomplish, for strangely enough basophilic vacuolation occurs in both hypo- and hyperthyroid conditions (Severinghaus *et al.*[18, 19]).

The study of the hypophyses of these massively injected dogs points still more clearly to the conclusion that basophilic vacuolation is a characteristic retrogressive alteration which the cells undergo when their normal physiology is disturbed. A previous suggestion that the Cushing syndrome is one of these disturbances now seems even more justified, and we are inclined to regard the Crooke changes as an aspect of the general granule liquefaction which also appears after castration or thyroidectomy.

Furthermore, a study of these glands again confirms one in the opinion that all of these basophilic changes are atrophic in character. This in no sense implies that the destruction of cytoplasmic granulation through liquefaction produces a substance which is hormonally impotent. There is, however, no good experimental evidence to indicate that the widespread vacuolation due to either castration or thyroidectomy is associated with any increase in the respective gonadotropic or thyrotropic hormone content of the anterior hypophysis. The increase of gonadotropic hormone which occurs after castration is much more logically related to the great increase of large granular basophils, while after thyroidectomy, if any increase in the thyrotropic hormone content occurs, which is very questionable, the same correlation is indicated.

Sizeable areas in the hypophyses of these dogs are composed of secretory active basophils. These cells cannot be identified by reference to the Golgi apparatus, for the Golgi region differences between the two types of chromophils in the dog's hypophysis are not clear-cut.[17] The cells, however, have the general characteristics of basophils. They are much larger than acidophils (Fig. 25). The cytoplasmic granulation is fine and stains a light slate blue color rather than the dark blue of the typical basophil. The mitochondria, though faultily preserved with Zenker-formalin, are numerous and the Golgi region is hypertrophied. These cytoplasmic features indicate a metabolically active cell, producing and liberating a secretory product at a rate above normal. The cells seem to have developed from the chromophobes which are

now entirely missing from such regions. It is not impossible that these basophils which are now in a phase of hyperactivity will later succumb to atrophic vacuolar changes.

There is some clinical evidence to indicate that patients with the Cushing syndrome pass through a period of hyperthyroidism which is followed by hypothyroid symptoms. This reversal of thyroid activity would produce correlated anterior lobe changes.

The changes which the acidophils of the injected dogs undergo are much less spectacular, but nevertheless real. If the proportion of acidophilic cells differs from the normal, it could be established only by statistical methods on a much larger series of animals. Suffice it to say that acidophils are abundant. From the fact that basophilic changes in these glands resemble in part those which follow thyroidectomy, the reader may have expected a great diminution or even an absence of acidophils. However, thyroidectomy in the dog does not result in the striking disappearance of acidophils which one sees, for example, in the anterior hypophysis of the rat (unpublished data, Severinghaus).

Acidophilic regions of the gland do not present a uniform appearance. In certain areas the cells are small and irregularly shrunken, with varying degrees of granulation. They have pyknotic nuclei which stain deeply basophilic (Fig. 27). Scattered among these acidophils are many chromophobic cells with exactly the same nuclear characteristics. These two types of cells are typical of stages in the reversion of acidophils to chromophobes in the cycle of secretion previously described in the human hypophysis.[21, 23] Such acidophils will revert to chromophobes but after a nuclear reorganization may at any time begin a new cycle of acidophilic activity. In this connection it is interesting to record that large areas of normal acidophils and chromophobes are also characteristic of the anterior lobe of these injected dogs (Fig. 26).

Finally, it may be pointed out that whereas the dog's anterior pituitary gland contains many small basophilic colloid bodies, they seem decidedly increased in these experimental animals. These masses are frequently surrounded by a row of cells so that they occupy the center of a cord of cells. At other times they appear along the connective tissue framework of the gland and are therefore between the cell cords. Ciliated cells have been seen on occasions to border upon larger colloid inclusions, but these

seem different in character from those described above. The origin and nature of the colloid remains obscure.

(*B*) *Anterior Hypophyses of Dogs Injected with Canine Antihormone*

A single glance at the anterior hypophyses of these dogs reveals that they have undergone striking alterations. Four features are especially striking: (1) the scarcity of typical areas of chromophobic cells; (2) the replacement of normal basophils by hyalinized or vacuolated cells, or by large sparsely granulated cells rich in mitochondria; (3) modifications in the acidophils; and (4) a marked hyperemia and edema of the gland.

The absence of the usual large number of chromophobes gives the anterior lobe a highly granular appearance. Chromophobes have not entirely disappeared, but they are greatly reduced and scattered individually throughout the glandular stroma, in contrast with the grouped arrangement of the normal gland. This unquestionably adds to the impression of their scarcity (Fig. 32).

The decrease in chromophobes is directly correlated with a marked numerical increase of large granulated cells of basophilic character. The coloration of these cells is extremely variable. It varies from the occasional normal dark blue of the normal basophil through varying shades of purple with an increasing reddish cast. Granulation is progressively coarser and more scattered in such a series of cells. There is little reason to question the basophilic lineage of any of these cells. The changes in coloration are due to varying degrees of degranulation of the specific basophil granules and a simultaneous increase in the mitochondrial content of the cytoplasm. With Zenker-formalin fixation the mitochondria have been preserved, although less perfectly than with the chrome-osmic fixation.

The staining of alternate 3 μ sections with copper hematoxylin clearly demarcates the acidophils and shows the varying degree of mitochondrial development in the basophils, since in the latter cells only the mitochondria of the cytoplasm stain. The cytoplasm of the dark basophils appears clear with this technic. In the purplish cells the scattered mitochondria granules stand out sharply against the clear cytoplasm. They have a coarse irregular aspect and are in no way suggestive of acidophilic granules.

The frequent presence of a large Golgi zone as well as the mitochondrial increase leads us to believe that these cells are active both in the production and in the release of a secretory substance. Their rapid increase in large numbers at the expense of the chromophobes indicates that there are many undifferentiated chromophobes which have basophilic potentiality or that chromophobes of the acidophil line are able to return to an undifferentiated state and then give rise to basophils. In the dog one is not able to differentiate the granular cells with accuracy by the shape and position of the Golgi apparatus, so that any attempt to analyze the chromophobes, as can easily be done in the rat,[17] is impracticable.

Many of the mature basophils show evidence of the onset of vacuolation as described in the previous section of this paper. An irregular and massive clumping of the specific cytoplasmic granules has occurred in most of the cells. The deep blue granular masses are sometimes distributed at random throughout the cell, but more frequently are concentrated centrally near the nucleus. Surrounding them is a non-granular, amorphous cytoplasm which exhibits a variable affinity for the dyes. In dog No. 4 the hyaline substance of the majority of these cells stains a pinkish slate color even after over-extraction of the acid fuchsin and the forcing of the aniline blue. The similarity of these cells to the hyalinized basophils of the human anterior lobe in cases of pituitary basophilism is most striking (Fig. 31).

The acidophils are present in such numbers as to suggest no numerical deviation from the normal. Many of the cells are small and compact and well granulated, but some are large and have a prominent Golgi zone. In many of these the granulation is more sparse. The nuclei are on the whole normal, there being no excessive amount of lobulation or pyknosis. Scattered among the normal acidophils are cells which stand out because of their brilliant fuchsin staining. These cells range in size from cells smaller than the normal acidophils to cells considerably larger. Their shape is often irregular. Cytoplasmic granules cannot be resolved. The nucleus is pyknotic and basophilic. These cells, plentiful in the antihormone serum-injected dogs, can be found by search in the dogs injected with pituitary extract for prolonged periods. Their numerical difference in the two groups is obvious, even with

a cursory examination of the slides. These atypical acidophils are not unlike cells to be described presently in the anterior lobe of the pituitary in sheep following prolonged injections of pituitary extract. In the sheep one can observe stages in the transformation of normal granular acidophils into these cells. In the dog one has no clue either as to their origin or significance.

The hypophyses of the antihormone serum-injected dogs show a marked hyperemia and edema. A careful microscopic study of these conditions has revealed some very interesting facts. The capillaries appear either as large sinusoids distended with blood cells, or as contracted capillaries of small diameter. These latter usually run through an edematous area which approximates the size of the most distended capillaries (Figs. 32, 33). In general, the edematous area is filled with a blue granular substance which has all the characteristics of basophilic granules. In fact, under the lower magnifications, one gains the impression that these extracellular granules are areas of basophilic cells. On numerous occasions the granules of such areas are directly continuous with the dispersing basophilic granules of bordering cells. This phenomenon can also be found at places along the distended capillaries (Fig. 30). It is common also to find blood cells within the edematous area outside the capillary wall. Since the tissue is excellently preserved, there can be but one interpretation, namely that the capillaries are in frequent communication with the edematous areas and that active degranulation of the cells is taking place into these areas as well as into the capillaries directly. The cytoplasmic granules are still recognizable in the blood plasma * (see Figs. 30 and 33).

(*C*) *Anterior Lobe of the Ewe Lamb after Prolonged Injection with Sheep Pituitary Extract*

The hypophyses to be described in this section are from 3 highly inbred ewe lambs 10 months of age. The 2 injected animals were twins, and the normal control a half-sister.

* For the sake of emphasis one of us (A. E. S.) wishes to state that during the last 10 years devoted largely to a cytological study of the endocrine glands, granular substances within the blood plasma have been noted on numerous occasions. On no occasion, however, have these been looked upon as cytoplasmic in origin, but rather as coagulation products of the plasma. In the present highly activated glands, however, we have not the slightest reservation concerning the identity of these granules.

The anterior lobe of the pituitary in sheep is normally predominantly acidophilic. Limited areas of basophils are found peripherally and may extend here and there as solid cords of basophilic cells into the deeper portions of the gland. In addition to these cords, there are scattered isolated basophils throughout the entire glandular area. As in all other species, the basophils may be found in varying degrees of degranulation but the majority of the cells have a cytoplasm well filled with distinct basophilic granules which stain brilliantly with aniline blue after Zenker-formalin fixation. The Golgi region is not sharply demarcated in the heavily granulated cells, but in the degranulating cells it is seen as a prominent cytoplasmic structure, somewhat acidophilic in coloration due to the abundance of mitochondria in this region.

The acidophils have a distinctly granular cytoplasm and a nucleus with a network of chromatin and one or two large nucleoli.

However, various modifications of the acidophils occur, and these again closely approximate the stages described for the secretory cycle in the acidophils of the human hypophysis (Severinghaus [21, 23]). In addition to nuclear and cytoplasmic granular changes, the acidophils of the sheep exhibit a great variety of shapes. Round, ovoid or polyhedral shapes are common. Frequently cells are elongated and it is not uncommon to find a whole row of elongate, almost columnar cells bordering a sinusoid. In a recent paper Warbritton and McKenzie [34] describe as many as nine types of cells in the ewe, in place of the traditional three. Among their criteria of classification are the shape of cells and the degree of granulation. We have found no cells in the anterior lobe of the pituitary in sheep which could not be recognized as either acidophil, basophil or chromophobe. Variability in the size and shape of glandular cells, which constantly occurs with the elaboration and discharge of secretory products by the cell, can hardly be acceptable criteria for separating cells into distinct types, in the sense that chromophobes, acidophils and basophils are separable.

One modification of the acidophil is deserving of special mention. Occasional cells are present with a homogeneous, nongranular cytoplasm which stains brilliantly with acid fuchsin. The nuclei of these cells are highly pyknotic and basophilic. The cells

are very irregular in shape. It is evident from cells in which granular and non-granular areas are associated in varying proportions that these hyalinized cells are modified acidophils.

The hypophyses of the twin ewes which were injected with an extract of sheep pituitary gland are clearly modified. The most striking change is an almost universal degranulation of the basophilic cells (Fig. 28). It requires considerable search to locate a normally granulated basophil. Mitochondria and the Golgi apparatus are prominent in the degranulating basophils. The acidophils seem increased in size, are compactly granulated, and stain more brilliantly than do the cells of the control. The cells with a brilliant acidophilic hyaline cytoplasm are much more numerous, as are the transitional stages in which a partial granulation still remains. Areas of small acidophils with pyknotic basophilic nuclei are common. These glands give cytological evidence in both chromophilic cells, but especially in the basophils, that the secretory activity of the anterior hypophysis is considerably increased over the normal.

Discussion and Conclusions

We are not able at this time to offer a thorough interpretation of the observations that have been described above. We may, however, emphasize what seem to us to be the more important observations. In the first place, the "Crooke changes" heretofore described only in the human pituitary gland have now been experimentally produced in dogs. Although individual cells with the Crooke change were found in the hypophyses of the dogs that had prolonged injections of anterior pituitary lobe extract, they were much more common in those dogs injected with the antihormone serum. Basophilic changes, characteristic of castration and thyroidectomy, on the contrary, were the outstanding characteristics of the former group presumably because of the longer period of injections.

The profound changes in the anterior lobe of the pituitary described in these experiments would have little interest or value unless we attempted to gain from them some insight into the physiological processes with which they are associated. In other words, the question of major importance is, "How are these cytological changes produced?" It is obvious that the proper correla-

tion of sufficient data of this character must eventually lead to a correct understanding of the glandular functions.

A number of possibilities immediately suggest themselves. In the first place, it is conceivable that the anterior lobe is being damaged by cytotoxins which the extended injections may call forth. Or again, the changes may be produced by the direct effects of the injected pituitary extract or indirectly through the increased secretions of other endocrine glands which the injections may have activated. In this connection it is necessary to know whether the effective principles are of anterior lobe origin or whether other pituitary hormones (antidiuretic, and so on), which we know to be present in the extract, are also involved. In the third place, the anterior lobe changes may be due directly or indirectly to the antihormones which have been elaborated. Finally, one must ask if the changes indicate the elaboration of the antihormone by the hypophysis itself.

Some of these questions seem rather easily disposed of. It is not likely that the antidiuretic principle is responsible for the gross cytological changes in the anterior lobes of these dogs. The hypophysis of the ewe injected with the sheep extract known to contain the antidiuretic hormone gave evidence of secretion activation but showed none of the profound basophilic changes seen in the dogs injected with the antiserum or the pituitary gland extract.

The evidence further indicates that the observed changes are not to be attributed to the actual elaboration of the antihormones by the pituitary glands. Considerable data are now available to show that the antihormones are produced in the body tissues even in the absence of the hypophysis. Our results contain nothing to lead us to question the assumption that the antihormones are produced in such a likely site, for example, as the reticuloendothelial system.

Direct experimental evidence seems difficult to obtain either for or against the supposition that damage to the hypophysis by the action of cytotoxins may result from prolonged injection of pituitary extract. The possible rôle of the Forssman reaction in this problem remains to be investigated.

The dissimilarity of the effect upon the dog and sheep hypophysis and upon other endocrine glands after similar prolonged

injections of pituitary extract makes it difficult to assume that the anterior lobe changes are due solely to the direct action of the injections. This fact likewise lessens the possibility that the injections activated the other endocrine glands which in turn produced the changes finally seen in the hypophyses. It is true that the initial effect upon the hypophyses in all of the animals here described has seemed to us to be a stimulation of secretory activity. We believe this to be due to an activation, by the injections, of the gonads and thyroid (adrenals?) whose hormones in turn affect the hypophysis. The anterior hypophysis of the injected ewe remains in this state of hyperactivity even after 6 months of daily injection. The antiserum-injected dogs have hypophyses which show in part signs of increased activity and in part evidences of retrogression, perhaps the result of a preceding exhaustion through abnormal activity. The pituitary-injected dogs alone show the widespread changes, especially in the basophilic cells, which combine the characteristics of the Cushing syndrome of castration and of thyroidectomy.

The early stages in the phenomena of basophilic vacuolation, which we regard as an indication of reduced rather than increased secretory activity, might be expected in the hypophyses of dogs injected with the antihormone serum. If the antihormones begin a neutralization of the hypophyseal hormones, as in the phenomena of immunization, then the thyroid, gonads and other glands would begin to suffer the effects of pituitary deprivation. The result of their progressively decreasing activity should eventually be an effect upon the anterior lobe simulating a total ablation of this organ.

Those anterior lobe features resulting from antihormone-serum injections, which we have interpreted above as evidence of increased hypophyseal secretion production and release, are more difficult to understand. We know that the earliest effects upon the hypophysis of total ablation of the thyroid or gonads are in part activating rather than depressing. This is shown by the great numerical increase of basophils at the expense of chromophobes. Cytologically, however, such cells proceed rapidly to vacuolation and do not give evidence of increased secretory release as do those large areas of basophils in the hypophyses of antihormone-serum-injected dogs. Moreover, we have no right to assume that by de-

pressing a gonad or thyroid we immediately completely inactivate it, and thus produce in the experimental animal a situation comparable to the earliest period of total glandular ablation.

It should be pointed out that many investigators believe in the activation of secretory processes in the pituitary gland by a suppression of the gonads. This view is based on a considerable body of evidence, especially that derived from certain parabiotic experiments and from a comparative analysis of the hormone content, supposedly of anterior lobe origin, in the urine of pre- and postmenopausal women. The evidence for a diametrically opposite view, which cytological studies in a wide variety of experimental procedures have confirmed without exception, has been presented elsewhere (Severinghaus [22]). Although progress is evident of a harmonization of these conflicting views, the matter is far from being settled. We need not be unduly exercised over these discrepancies, but that we recognize them is of first importance. Discrepancies are the rich deposits of discovery. They indicate the points at which our efforts should be redoubled.

One great difficulty in attempting to interpret the results of the present experiments lies in the fact that massive dosages have been employed. Errors may readily be committed by attempting to impute the normal physiological glandular interplay to experiments in which the whole organism may have been thrown into a state of physiological disorder by an intolerable, massive administration of a certain potent hormone. In this condition it is conceivable that the activity of a gland may be altered quantitatively and qualitatively, and that further complications arise if, at the same time, the end organs lose their capacity to respond normally.

Fully realizing the difficulties involved, we venture to go beyond a mere recital of our cytological findings and state that to us the most reasonable interpretation of the cytological changes in the anterior hypophysis, due to prolonged administration of pituitary extract, is obtained by assuming that the following reactions occur in sequence in the experimental animal:

1. Injection of pituitary extract activates the endocrine glands related to the anterior lobe, namely, gonads, thyroid, and probably adrenals.

2. The increased secretion of these activated glands in turn stimulates the anterior lobe which augments with its own secretion

the injected extract. This was seen in the case of the treated ewes.

3. The excess of extract of the anterior lobe of the pituitary from a foreign species results in the development of a humoral resistance (immunity) which manifests itself by the production of antihormones.

4. The antihormones at first neutralize the injected pituitary extract, and later the secretion of the hypophysis of the injected animal itself. Thereafter the end organs (gonads, thyroids, adrenals) react by atrophy as in states of pituitary insufficiency, even though the hypophysis continues its activity.

5. The atrophy of these related glands in turn brings about in the anterior lobe certain changes which are characteristic of gonadectomy and thyroidectomy (adrenalectomy?). A combination of these effects well describes the condition actually observed in the anterior lobe of the pituitary.

The Crooke changes, usually associated with hyperactivity of the adrenal cortex, but also with hypothyroid and hypogonad states are most closely simulated in the antiserum-injected dogs in which the adrenals are not greatly altered.

Although the animals exhibit some of the characteristics of hypophysectomy, it is evident that injections have accomplished a relatively complete thyroidectomy without surgical interference. The lowered basal metabolic rate, hypercholesterolemia, and the histological appearance of the pituitary are proof of this fact. The atrophic thyroid gland and the above facts contribute proof that in this case function and histological appearance in the thyroid were also correlated.

One cannot say from the experiment whether the hypophysis which results from prolonged pituitary extract injection and which shows the changes normally following thyroidectomy and gonadectomy has been inactivated, or whether a normal or even increased output of secretion is taking place. Regardless of the rate of secretion release, the hormones are constantly being neutralized by the circulating antihormones. On cytological grounds it can be said that the hypophysis continues some of its secretory activity. Certain of its products may be inactivated by the antihormones, so plentiful in the blood, while others may be unaffected, thus resulting in a state not entirely comparable to hypophysectomy.

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DESCRIPTION OF PLATES

PLATE 67

None of these photographs is retouched. They were taken at a magnification of about 950 ×, unless otherwise designated.

FIGS. 1–4 show the clumping of basophilic granules into irregular masses. The dark masses are large colloid vacuoles. The clear vacuoles are also seen in various stages in Figs. 2 and 4.

FIGS. 5–9 inclusive show various stages of the transitional formation of dark granular masses into colloid vacuoles. Note their general distribution throughout the cytoplasm. The cell borders frequently become indistinct.

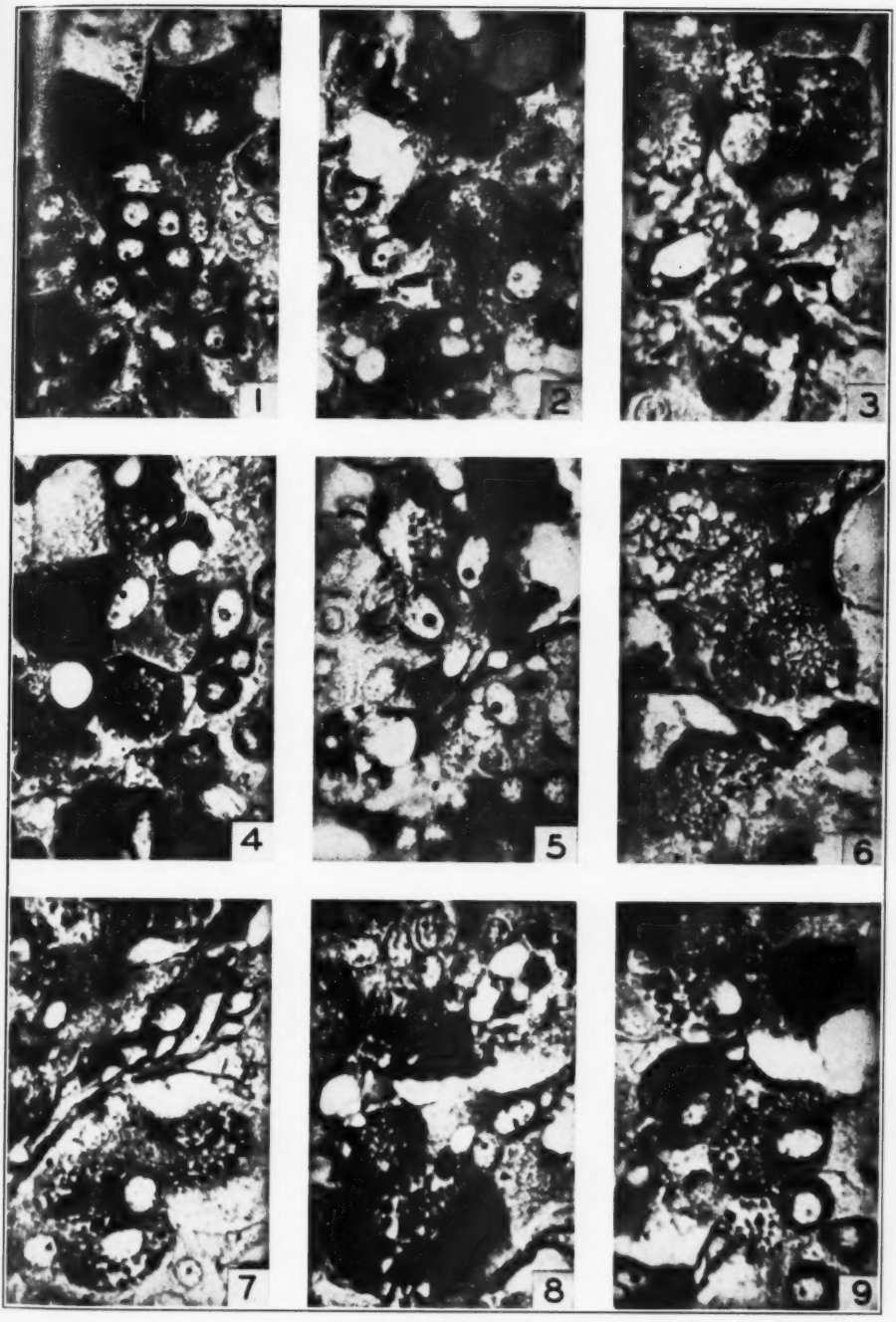
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Plate 68

Figs. 10–12. These photographs show the presence of larger dark basophilic vacuoles and their formation from smaller ones. See especially cell in Fig. 12. Cells in the lower left of Fig. 10, and upper and center right of Fig. 11 show vacuoles of the paler basophilic substance.

Figs. 13–16 inclusive show the association of all 3 types of vacuoles within a single cell. In Fig. 15 one large disintegrating basophil (chromophobe indenting on right) occupies almost the entire area below the capillary and to the right of the connective tissue fibers.

Fig. 17. A typical castration type of vacuolation is seen in the cell at the upper center.

Fig. 18. Large basophils whose borders are indistinct border on the sinusoids. Such basophilic masses of cells are common.

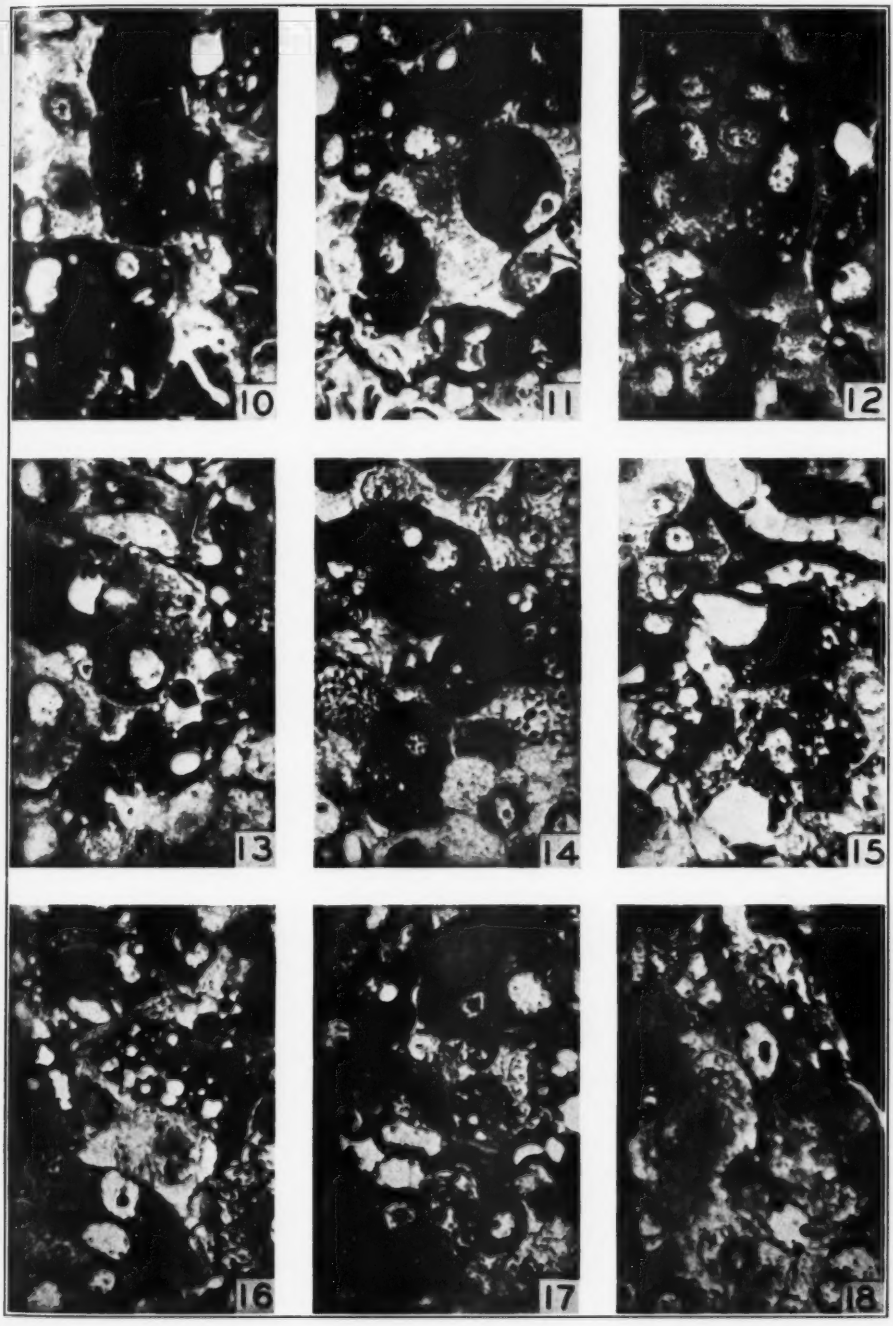
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PLATE 69

FIGS. 19–24 show many stages in the progressive development of the clear vacuoles described in the text. With these are associated vacuoles of the other 2 types. These cells are typical of thyroidectomy changes in the basophils of the dog.

FIG. 25 shows a typical group of smaller basophils with fine cytoplasmic granulation and abundant mitochondria. These appear cytologically to be very active cells.

FIG. 26 shows a characteristic region of normal appearing acidophils.

FIG. 27 shows a characteristic region of pyknotic nucleated acidophils, an indication of cyclical secretory activity in the acidophils. See text.

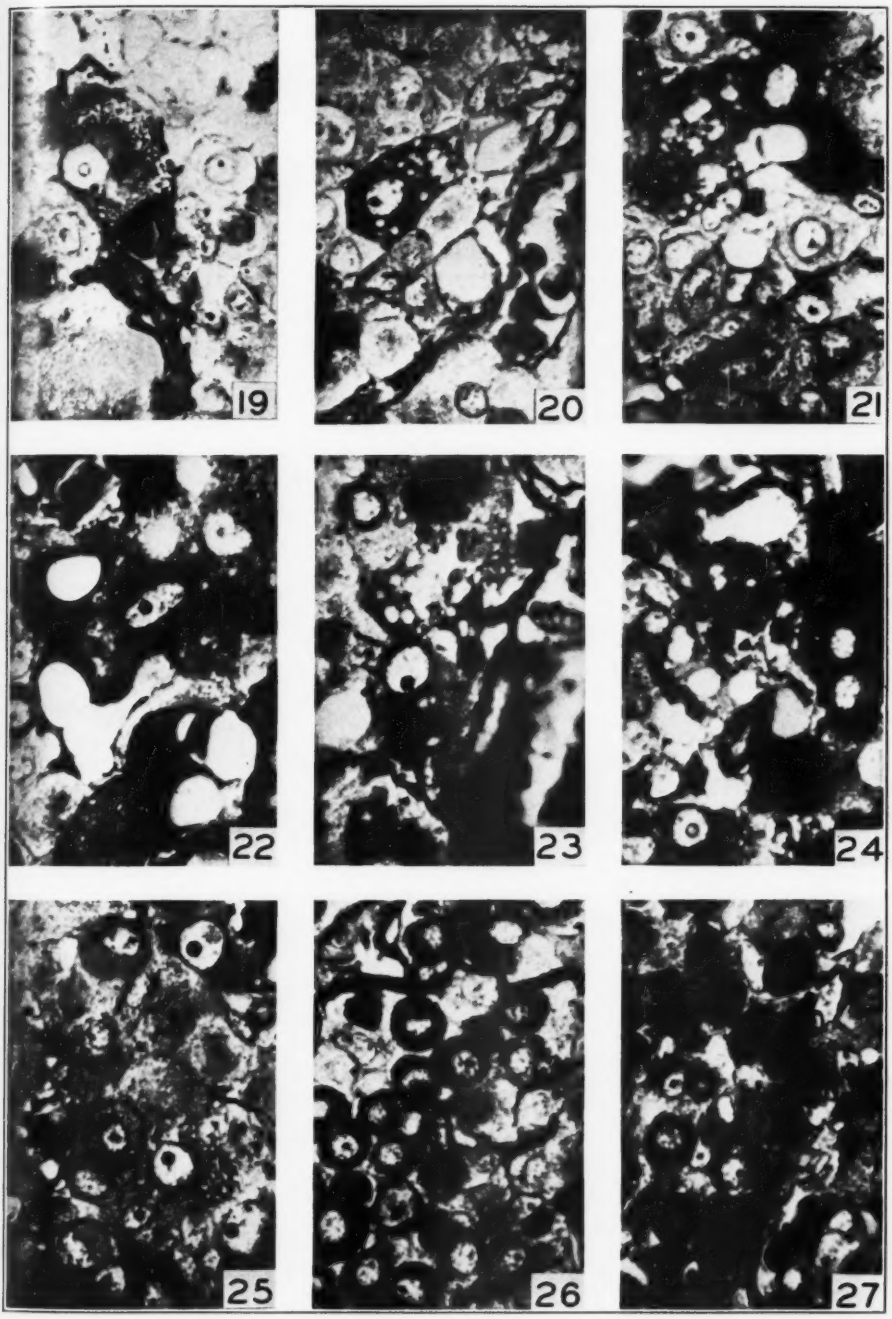
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PLATE 70

FIG. 28 shows a typical area from the anterior hypophysis of a 10 months old lamb injected daily for 6 months with sheep pituitary extract. The dark granulated cells are acidophils. Homogeneous black areas in the cells are hyalinized portions of the cells. The light areas are largely degranulated basophils with a sparse grayish cytoplasmic granulation. About × 550.

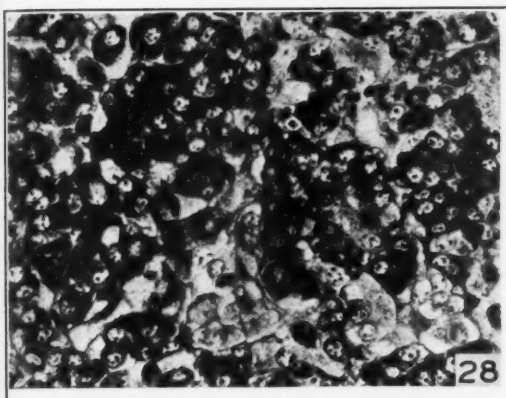
FIG. 29 shows a control lamb hypophysis. Note the distinct basophilic (deep blue) granulation of the basophils. Only color photography could indicate the marked contrast of these two glands. About × 550.

FIG. 30 shows actual degranulation of the bordering basophils into a distended sinusoid. Note the granular substance continuous across the sinusoid wall just to the right of the large central basophil. About × 2000.

FIG. 31. The large basophil in the center has cytoplasmic granulation adjacent to and below the nucleus, but the periphery of the cell has been completely hyalinized. The striking similarity to Crooke changes is obvious. × 2000.

FIG. 32. Typical area of a pituitary from an antihormone-injected animal. Note the extreme hyperemia and edema. A distended sinusoid is seen at the upper right, while at the lower right and left are typical contracted capillaries within edematous areas. Note the extravasation of red cells above the capillary in the edematous area and at the left center of the figure.

FIG. 33. High power field of square in Fig. 32. In the upper left the capillary, with its distinct endothelial nuclei, lies in an edematous space which is filled with red blood cells. Numerous examples such as this give evidence of widespread communication of the capillaries with the spaces. Note that the distended capillaries approximate in a cross section area the edematous spaces.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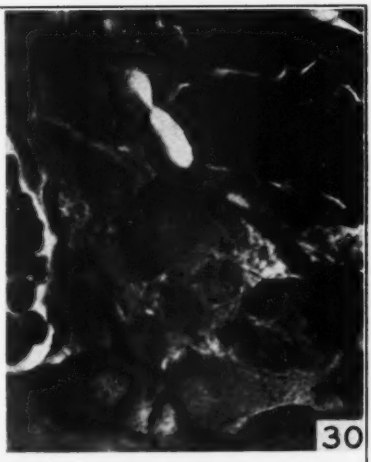
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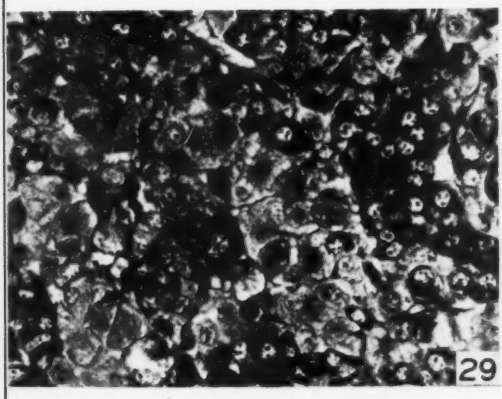
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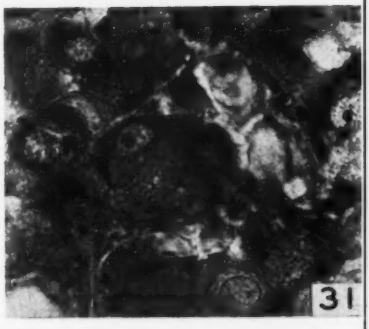
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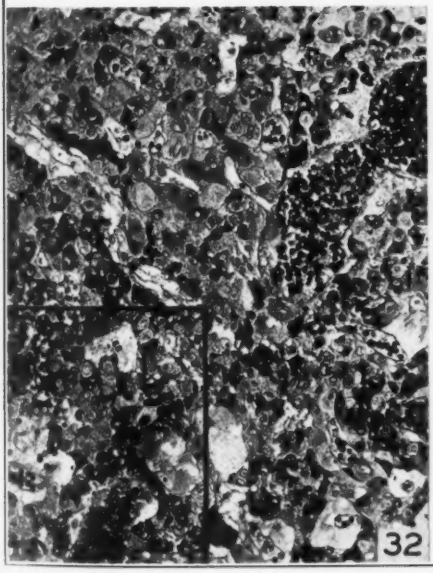
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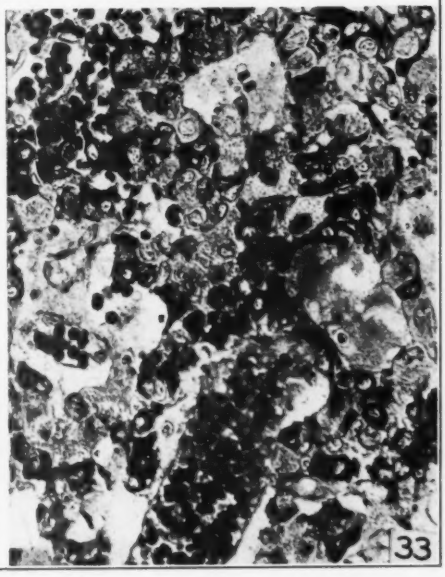
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THE LYMPHOCYTE IN ACUTE INFLAMMATION *

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INTRODUCTION AND REVIEW OF THE LITERATURE

The attitude of many histologists and pathologists toward the function of the lymphocyte is excellently summarized by Rich [1] in his general review of inflammation in resistance to infection. He states: "I am sure that all who are engaged in the study and teaching of pathology will agree that the complete ignorance of the function of this cell is one of the most humiliating and disgraceful gaps in all medical knowledge. . . . Literally, nothing of importance is known regarding the potentialities of these cells other than that they move and that they reproduce themselves."

The explanation of the rôle of the lymphocyte in tissue reactions accepted currently by teaching pathologists may be illustrated by a quotation from the latest edition of Boyd's Textbook of Pathology [2]: "In chronic inflammation and in the later stages of acute inflammation the lymphocyte may be the main cell of the exudate. The cells of such collections are often called by the non-committal name of 'small round cells.' This term is conveniently non-committal as to the origin of the small cells. It appears probable that the majority of the small round cells are derived from the tissues rather than from the blood. The lymphocyte of the blood has very little cytoplasm, and is therefore only slightly amœboid and not at all phagocytic for bacteria. It migrates from the vessels with difficulty, and much later than the polymorphonuclears. It is rather remarkable that we are so ignorant as to the exact rôle played by a cell which plays so dominant a part in the chronic infections."

MacCallum [3] in his textbook of pathology writes: "The mononuclear cells, which cannot be recognized as lymphocytes, are larger and assume a great variety of forms and sizes, so that they may come to be veritable giant-cells, often with many nuclei, and still one can draw no sharp line anywhere to divide them into

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groups. Maximow, indeed, was sure that they all grew out of the lymphocytes which had emigrated from the blood-vessels. They are normally present in a great many places, not assembled in definite nodules of coherent tissue, as in the lymph-nodules, but scattered loosely in the mucosa of the whole intestinal tract, in the adventitial tissue of blood-vessels, in the loose tissue about the bronchi or the ureters — indeed, in the loose tissue anywhere in the body. At any rate they arrive very promptly in great numbers in any such tissue, if an occasion arises in which they are required to carry on for a time a phagocytic activity — to clean up the débris of cells. They are not easy to describe. From their motions when alive they seem very different from lymphocytes and it is hard to believe that those sluggish cells could have grown into these which reach out so eagerly and swallow fragments of cells so greedily. When they are fixed and stained they lose these characteristics and appear as rather large cells growing somewhat paler as they increase in size with vesicular nucleus with scattered chromatin particles. They are evidently the same cells as the monocytes of the circulating blood and they are the cells which have been variously called clasmatocytes, adventitial cells, macrophages, histiocytes, reticulo-endothelial cells, endothelial leucocytes, polyblasts, etc. We have hitherto called them mononuclear wandering cells and, since this is quite non-committal, we may well go on with it."

Muir [4] states: "It can hardly be said that they (lymphocytes) have no phagocytic power, but this is at a minimum. Probably they become enlarged when they are going to exert their function. . . . They probably represent a response to the mildest type of irritation, but their precise function is not known. They migrate but are more concerned with chronic inflammation."

We suggest that the uncertainty of the pathologists finds its basis in the usually confusing and often indefinite statements found in the histology books on the potentialities and functions of the lymphocyte. Cowdry [5] writes: "Emigration of lymphocytes takes place in a wide variety of conditions which have the single feature in common that they are of longer standing (chronic) and less temporary (acute) than those which lead to the mobilization outside of blood vessels of neutrophiles." Bailey [6] states: "The lymphocytes migrate through the walls of the capillaries and dis-

play marked motility in the connective tissue. Their function is not clear but apparently they can develop into plasma cells and monocytes." Bremer [7] concludes that "lymphocytes are only infrequently phagocytic to vital dyes."

A second reason for the uncertainty of pathologists regarding this question is their lack of correlation of morphological modification of the lymphocyte with its functional variability. Aschoff [8] in discussing the ability of the lymphocyte to hypertrophy and become a phagocyte said, "I can only say there is no certain proof for such a transformation."

Metchnikoff,[9] probably because he was confronted with less complex situations than Aschoff, assumes a more familiar attitude toward these cells which is reflected in his statement: "The smaller white corpuscles found in fairly large numbers in the blood and the lymph and which are commonly known as lymphocytes or small lymphocytes are simply leucocytes with very little protoplasm which in this state never fulfill phagocytic functions. It is only when it becomes older, when its nucleus, single and rich in chromatin, becomes surrounded by an ample layer of protoplasm, that the lymphocyte becomes capable of ingesting and resorbing foreign bodies. Several authors, with Ehrlich at their head, still assign to these larger cells the same name — lymphocytes. Others however, give them the name of large mononuclear cells. Confusion is thus possible, especially as Ehrlich includes under the same term the large mononucleated leucocyte, a very rare form of cell in human blood which is distinguished by the greater staining capacity of its nucleus. To avoid this inconvenience I propose to designate the large lymphocytes by the name of blood macrophages and lymph macrophages (haemomacrophages, lymphomacrophages). . . . The mesoblastic phagocytes of the vertebrata are divided then, into fixed phagocytes — the macrophages of the spleen, endothelia, connective tissue, neuroglia, and muscle fibers — and free phagocytes. These latter are sometimes haemo- or lymphomacrophages, sometimes microphages. The fixed macrophages and the free macrophages resemble one another so greatly that it is very often extremely difficult, if not impossible, to differentiate them. For this reason it is often very useful, when the exact origin of a large phagocyte is not known, simply to name it macrophage."

Since Metchnikoff's description of the transformation of the lymphocyte to a macrophage much experimental morphological confirmatory evidence has accumulated.

Maximow [10–17] studied the problem of the function of the lymphocyte very extensively. His views are expressed in the following quotation from his Textbook of Histology. "The question of the prospective potencies of the small lymphocytes is of special interest. Many investigators believe these cells to be specifically differentiated elements incapable of further development. It has been conclusively shown, however, that some of the polyblasts in all inflammatory lesions arise from the local fixed macrophages, but that a much more abundant and important source is the lymphocytes and monocytes of the blood. These agranulocytes migrate from the blood vessels into the tissue, undergo here a rapid hypertrophy and are transformed into large phagocytic elements. In the first two days after the onset of inflammation they can still be distinguished from the polyblasts of local fixed macrophage origin by their smaller size. But, as they continue to increase in size, after two days or more the polyblasts of local and of hematogenous origin can no longer be distinguished. The differentiation of the polyblasts from the two sources becomes all the more difficult as the hypertrophied lymphocytes and monocytes very soon begin to store vital dyes if the inflammation occurs in a vitally stained animal."

Among the investigators who have confirmed the observations of Metchnikoff and Maximow, we wish to note the following: Ziegler [18] who concluded in his studies of edema of the skin and subcutaneous tissues that lymphocytes wander out of the blood and lymph vessels into an area of inflammation where they undergo morphological changes and become phagocytic; Schwarz [19] who noted that 2 to 10 hours after the onset of an acute inflammatory process the lymphocytes made up a part of the cellular exudate and later transformed into large mononuclears; Helly [20] who, after studying the morphology of exudate cells in acute inflammations produced experimentally by the anthrax bacillus, staphylococcus, streptococcus, the typhoid and the colon bacillus, cholera vibrion, diphtheria bacillus, pneumococcus and the tubercle bacillus, concluded that the heterophils formed the microphages and the lymphocytes formed the macrophages in the inflammatory exu-

dates; Fischer [21] who found that about 7 hours after the introduction of irritants into the connective tissues of rats and mice, the lymphocytes that migrated into the area were changing into polyblasts; Tschaschin [22, 23] who supported Maximow's observations to the letter; Downey [24] who demonstrated that lymphocytes would take up colloidal dyes if the dyes were available; Bergel [25] who showed lymphocytes to be phagocytic for fats injected into the peritoneal and pleural cavities of guinea pigs and rabbits; Danchakoff and Seidlin [26] who observed that in the mesenchymal plate of the tail of a tadpole into which edestin was injected, lymphocytes migrated from the blood vessels and hypertrophied and gradually transformed into typical histotopic wandering cells or lymphoid phagocytes; and Stilwell [27] who observed inflammatory processes in the living frog's tongue. She injected diluted India ink intravenously and then injected egg yolk into the tongue and watched the local inflammatory process. The lymphocytes migrated at about 7 hours, hypertrophied and became phagocytic. Lang [28] concluded from a series of experiments on acute inflammation that lymphocytes and monocytes hypertrophy and become polyblasts. Bloom [29, 30] observed lymphocytes taken from the thoracic duct lymph transform into polyblasts between 7 and 20 hours after they were cultivated in tissue culture. Michels and Globus,[31, 32] in a study of the "so-called round cell infiltrations," found transitions between lymphocytes and macrophages. Watson [33] observed transitions from lymphocytes to phagocytic cells in the tissues of a patient dying of histoplasmosis. Ekola [34, 35] also demonstrated that lymphocytes transformed into macrophages. She studied connective tissue reactions resulting from the subcutaneous injection of various irritants such as sodium ricinoleate, trypan blue, diphtheria toxin and diphtheria soap vaccine. As early as 9 hours after the onset of inflammation she found lymphocytes changing into polyblasts. In the 1 and 2 day stages in her series polyblasts were easy to find. Higgins and Palmer [36] concluded that the lymphocytes could differentiate into histiocytic elements (macrophages) in experimentally produced hematomas. Stieve [37] observed that lymphocytes furnish part of the macrophages in the walls of inflamed human uteri, and Silberberg [38] found lymphocytes forming polyblasts (macrophages) in experimentally aseptically inflamed connective tissue.

From a survey of the above literature it is evident that an important part in the formation of the acute inflammatory cellular exudate has been ascribed to the lymphocyte. However, this function of the lymphocyte is not generally accepted by the authors of the current textbooks of pathology. We suggest this discrepancy is the result of the observation by pathologists of only the later stages of the acute inflammatory process at which time cell lineage is no longer discernible. Bloom [39] pointed out that since acute inflammation is such a dynamic phenomenon, it must be studied with dynamic technics. Above all, the first few hours after the onset of an inflammatory reaction must be studied carefully in order to obtain a true picture of the genesis of the process.

Materials and Methods

We present a series of experiments which, while adhering to Bloom's tenets, employ an original technic that allows the simultaneous demonstration of histogenous and hematogenous cellular elements contiguous in respect to time and environmental variation during the process of inflammation.

Young adult rabbits were used in our experiments. The hair was removed from the sides of the animals by shearing followed by shaving. A chemical depilatory was purposely avoided because of the possibility of cutaneous irritation.

An initial control biopsy is made prior to the injection of the irritant.

The irritant is injected subdermally at points 2.5 cm. to 4 cm. apart in the bare area. Egg albumin was found to be the most satisfactory irritant for our experiment. The optimal amount to inject was found to be from 40 to 80 ml. ($mm.^3$). Greater amounts than this produce excessive edema which interferes with subsequent preparation of removed tissues. Amounts less than this do not produce a maximal cellular response. The point of injection is marked by a bland skin stain, such as methyl violet, because the inflamed areas are difficult to find. A map of the injected area is made and the position and time of the injection is recorded.

Adequate sampling of the inflamed tissue for biopsy can be obtained by removal of areas 1, 2 and 4 hours after injection. Tissue is then removed at 4 hour intervals through the 1st day,

and subsequently daily biopsies are obtained until healing is complete.

Under aseptic conditions the skin and subcutaneous tissue over the inflamed area is incised. A small amount of the loose connective tissue is picked up with forceps and excised. This tissue is transferred to a slide, spread out into a thin layer with teasing needles and dried quickly by whipping through the air. Several spreads may be made. The skin and subcutaneous tissue are then sutured.

These tissue spreads from now on are treated like blood smears. A May-Grünwald-Giemsa staining combination is employed: 30 drops of the May-Grünwald stain are put on the spread for 1 minute. This stain is then diluted with an equal number of drops of distilled water buffered to pH 6.4 and allowed to remain 4 minutes. The slide is then drained and flooded with the Giemsa stain 1.5 strength (1.5 drops of Giemsa stain per 1 cc. of the buffered water). This remains for 8 minutes when the preparation is differentiated in distilled water and blotted dry.

The advantages of these dry spreads of the loose connective tissue over wet spreads or sections are the same as those pointed out by Kirschbaum and Downey [40] for hematopoietic tissues, namely, (1) a marked improvement in cytological detail, and (2) a basis of comparison between these and the cells seen in dry smears of blood. The disadvantage of this method is that in the tissue spreads areas may be too thick and some selection of fields is required. Also, cell structure is retained at the expense of tissue architecture.

Our technic may be summarized as follows: Successive biopsies of inflamed tissue are fixed by drying in the air and are then stained by the methods commonly employed in hematological studies — staining methods that give the best cytological detail and allow a comparison of recognizable hematogenous elements with histogenous elements.

Results *

We wish to limit ourselves as far as possible in this report to the behavior of the lymphocyte in the inflammatory process. We

* The morphological studies discussed were made under the supervision of Dr. Hal Downey.

recognize that the initial response of a tissue varies somewhat with the character of the foreign body producing the inflammation. Egg albumin with colloidal carbon or starch precipitates a deluge of pseudoeosinophils (polymorphonuclears) in the rabbit. However, egg albumin alone produces an essentially characteristic and less complex reaction and is, therefore, more satisfactory for following the lymphocytic response to inflammation.

In a morphological study of the cellular constituents of an inflamed area in connective tissue the histogenous and hematogenous components must be considered separately. The histogenous cells are largely clasmatocytes and fibroblasts which appear in approximately equal numbers together with occasional wandering lymphoid elements. Ranvier [41] introduced the name clasmatocytes for the potential phagocytes of the connective tissue. These cells were called fixed macrophages by Metchnikoff,[10] rhagiocrine cells by Renaut,[42] adventitial cells by Marchand,[43] pyrrhol cells by Goldmann,[44] resting wandering cells by Maximow [11–14, 17] and histiocytes by Aschoff and Kiyono.[45]

The hematogenous cells seen are granular leukocytes including the pseudoeosinophils (polymorphonuclears), the eosinophils and the basophils, as well as the non-granular leukocytes consisting of lymphocytes and monocytes.

The cells that receive our attention in this study are the clasmatocytes and lymphocytes, both of which can assume a phagocytic function.

We present our results by describing a series of cells from tissue removed for biopsy taken in a time sequence from experimentally produced inflammatory areas in rabbits.

The initial control biopsy contains clasmatocytes (Fig. 1). These are large round, oval or elongated cells. With our technic the nuclei of these cells show a rather coarse, sieve-like chromatin pattern. The chromatin granules are usually of a uniform size. However, occasionally condensation occurs resulting in the formation of clumps. The lavender parachromatin is sharply demarcated from the purple chromatin. The nuclear membrane is relatively thick and nucleoli occur infrequently. The nucleus is surrounded by a mildly basophilic, mottled, poorly outlined mass of cytoplasm. The granular basophilic spongioplasmic network contains many small, clear hyaloplasmic vacuoles. In some of these cells small

acidophilic and darkly basophilic cytoplasmic inclusions are seen. Occasionally in the cytoplasm large vacuoles are encountered.

The first 12 hours following the initiation of the acute inflammatory process the histogenous macrophage response overshadows the activity of the hematogenous macrophages. In this same period the activation of the clasmatocytes is accomplished. During the process of clasmatocytic activity there is little discernible change in nuclear architecture, so our description is largely concerned with cytoplasmic changes. Two hours after the injection of egg albumin the clasmatocytes (Fig. 2) show numerous large hyaloplasmic vacuoles containing various sized acidophilic inclusions. Although the extracellular albumin stains basophilic with the May-Grünwald-Giemsa combination, we assume the acidophilic cytoplasmic inclusions in the clasmatocytes are ingested egg albumin. From this stage on the clasmatocytes will be referred to as histogenous macrophages since this term indicates their source and function. We note that the number of clasmatocytes at this time has not increased.

At the 2 hour stage the hematogenous cellular response is indicated by the appearance of an occasional pseudoeosinophil, a cell homologous to the neutrophil of man. However, it is obvious that the first line of defence is by histogenous rather than hematogenous elements and the cell to react first is the clasmatocyte.

At the 4th hour of acute inflammation the tissue macrophages show the same changes as at the 2nd hour. The cytoplasm contains acidophilic inclusions of various sizes in great quantities. The hematogenous cells are slightly increased in number between the 2nd and 4th hours.

Eight hours after exposure to albumin the histogenous macrophages contain metachromatic granules in addition to the acidophilic cytoplasmic inclusions. Because of transition stages present, these granules are assumed to be a further step in the digestion of phagocytozed albumin. Migrating non-granular blood cells are beginning to appear after 8 hours of inflammation.

The 12th hour of the inflammatory process shows an increasingly frequent presence of metachromatic inclusions in the cytoplasm of the histogenous macrophages. Now for the first time the blood cells are beginning to approach the tissue cells in respect to numbers present. The pseudoeosinophil is a common cell and an

occasional eosinophil is seen. However, while the non-granular leukocytes, especially the lymphocytes, are not as conspicuous as the granular leukocytes, they are as numerous.

By the 14th hour (Fig. 3) the hematogenous elements outnumber the histogenous cells in the inflamed area, and the most frequently appearing hemic leukocyte is the lymphocyte. The histogenous macrophages still retain the typical clasmatocyte type of nucleus. Their cytoplasm still contains large numbers of acidophilic and metachromatic inclusions.

The lymphocytes (Fig. 8a) are small, medium and large. They have the characteristic pachychromatic nucleus in which the chromatin-parachromatin separation is not distinct. The narrow rim of cytoplasm in these cells is intensely basophilic due to a relatively small amount of yellow hyaloplasm as compared to the blue spongioplasm. Azurophilic inclusions are sometimes found in the hyaloplasm.

At this time — 14 hours after induction of the inflammatory process — indications of the lymphocyte transformation are well established. The pachychromatic lymphocytic nucleus becomes more diffuse. The chromatin becomes sharply demarcated from the parachromatin which shows a relative increase in amount. The chromatin blocks fragment and the nucleus assumes a more leptochromatic appearance. The end result of this transformation is the development of a small mononuclear cell whose nucleus bares little indication of its ancestry. This then — between the 8th and 14th hour — is the critical period in the evolution of the hematogenous exudate in acute inflammation. Any exposition of the inflammatory process which disregards the only opportunity to determine cell ancestry is open to serious criticism. At 8 hours the inflamed area shows many typical small lymphocytes — by the 14th hour all stages of transition between the trachychromatic lymphocyte nucleus to the more amblychromatic nucleus common to hematogenous macrophages are present.

The cytoplasm of the lymphocyte increases in amount and becomes less basophilic. This is brought about by an increase in the hyaloplasm with a resultant dispersal of the spongioplasm. Cells still containing azurophilic granulation may show a phagocytozed erythrocyte, acidophilic albuminous droplet or a degenerated pseudoeosinophil. Some of the metamorphozing lymphocytes show

small cytoplasmic pseudopodia. Since these cells have lost their pachychromatic nucleus and intensely basophilic cytoplasm — characteristics on which the recognition of lymphocytes depends — we shall refer to them as hematogenous macrophages (Fig. 8b).

At 18 hours (Fig. 4) the histogenous macrophages do not show any further changes. These cells are outnumbered by the hematogenous macrophages. The hematogenous macrophages are round or oval, moderately basophilic cells, much smaller than the histogenous macrophages. The nuclear and cytoplasmic hypertrophy is more marked than formerly and the phagocytic activity of these cells is more evident (Fig. 9).

The nucleus shows a somewhat coarser pattern in the hematogenous macrophage at this time than the nucleus of the histogenous macrophage.

By 26 hours (Fig. 5) the fibroblasts are showing mitosis. The histogenous macrophages have digested their acidophilic inclusions and consequently show only the metachromatic granules. Their vacuoles have decreased in size and the spongioplasm being more compact gives the cell a basophilic appearance.

The hematogenous macrophages now have a chromatin pattern similar to that of histogenous macrophages, but similarity to the lymphocyte nucleus is not apparent (Fig. 10). The cytoplasm of the hematogenous macrophages is still increasing and cell inclusions — vacuoles, albumin, pseudoeosinophils, and so on, are seen.

At the 49th hour (Fig. 6) the fibroblasts showing mitosis are the most conspicuous cells. However, they show no tendency to form definitive macrophages. The histogenous macrophages are becoming more basophilic and somewhat smaller. The presence of metachromatic granules indicates a previous phagocytic activity. The hematogenous macrophage nucleus does not show further changes. The cells are still smaller than the histogenous macrophages.

At 76 hours (Fig. 7) the histogenous and hematogenous macrophages approximate each other in size and morphology. They are both large mononuclear cells with comparatively leptochromatic nuclei and basophilic cytoplasm. Both cell lines show phagocytosis of erythrocytes, pseudoeosinophils, and so on (Fig. 11).

Discussion

Both acutely and chronically inflamed tissues possess a positive chemotactic attraction for hematogenous lymphocytes. In acute inflammatory exudates, in contrast to their retained lymphocytic morphology in chronic infiltrations, the lymphocytes become macrophages.

The transformation of a lymphocyte to a macrophage involves a marked alteration in nuclear structure as well as cytoplasmic and functional modifications. The factors underlying these changes are not clear. However, we wish to point out that the transformation of the pachychromatic lymphocytic nucleus to the larger, more leptochromatic nucleus of the macrophage, would not be incompatible with the development of a nuclear edema. The change in the nucleus is an increase in parachromatin and a decrease in size of the chromatin blocks. Even the increased chromatin-parachromatin definition could be produced by an increase in the nuclear sap, with a subsequent less dense chromatin. This edema we would associate with the disturbance of osmotic pressures due to a change in the pH of the intercellular fluids associated with acute inflammation. Certainly, in chronic lymphocytic infiltrations where these changes are less marked the lymphocyte nucleus retains its identity.

The cytoplasmic changes in the lymphocytes are the result of two not intimately associated processes. There is an increase in the hyaloplasm which may be the result of imbibition which by separating the particles of the blue spongioplasm gives us a cell with a moderate increase in cytoplasm with a decreased basophilia (Figs. 8, 9, 10). The cytoplasm of the hematogenous macrophage undergoes a quasihypertrophy by the phagocytosis of pseudoeosinophils, erythrocytes or albumin (Figs. 11, 12). It is only when the cytoplasmic edema is accompanied by phagocytosis that these cells obtain their maximum size.

Our studies tend to confirm the observation of Hertzog [46] that the phagocytic ability of a cell varies with its cytoplasmic mass.

We have in part at least answered Opie,[47] who in 1910 wrote: "If it were possible to define the origin of the mononuclear cells concerned in the inflammatory reaction of all vertebrate animals as well as it is possible to define the character and sources of the

common polynuclear leucocytes concerned in the same phenomenon, it might be possible to describe with an accurate generalization the essential nature of the cellular accumulation which follows the action of substance foreign to a tissue."

Conclusions

1. The transformation of clasmatocytes to histogenous macrophages is the initial response of the rabbit in acute inflammation.

2. The majority of the macrophages in the exudate associated with the acute inflammatory process are of hematogenous origin.

3. The lymphocyte-macrophage transformation occurs early in the course of the inflammation. By the 14th hour the lymphocytic origin of many mononuclear cells in an inflamed area is largely obscured. In studies made 18 hours or later after the onset of an acute inflammation in a tissue, cell lineage cannot be traced.

4. The employment of tissue spreads, dried and stained like blood smears, allows a comparison of the cells in an acutely inflamed tissue with cells of blood smears.

Note: I am indebted to Mr. Henry W. Morris for the microphotographs.

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DESCRIPTION OF PLATES

PLATE 71

FIG. 1. Normal clasmatocytes from the subcutaneous connective tissue. × 1100.

FIG. 2. Biopsied connective tissue during acute inflammatory stage at 2 hours. Activated clasmatocytes showing phagocytosis (histogenous macrophages). × 1200.

FIG. 3. Acute inflammatory stage at 14 hours. a = Histogenous macrophage; b = lymphocytes. × 1100.

FIG. 4. Acute inflammatory stage at 18 hours. a = Histogenous macrophage; b = hematogenous macrophages (hypertrophied lymphocytes). × 1100.

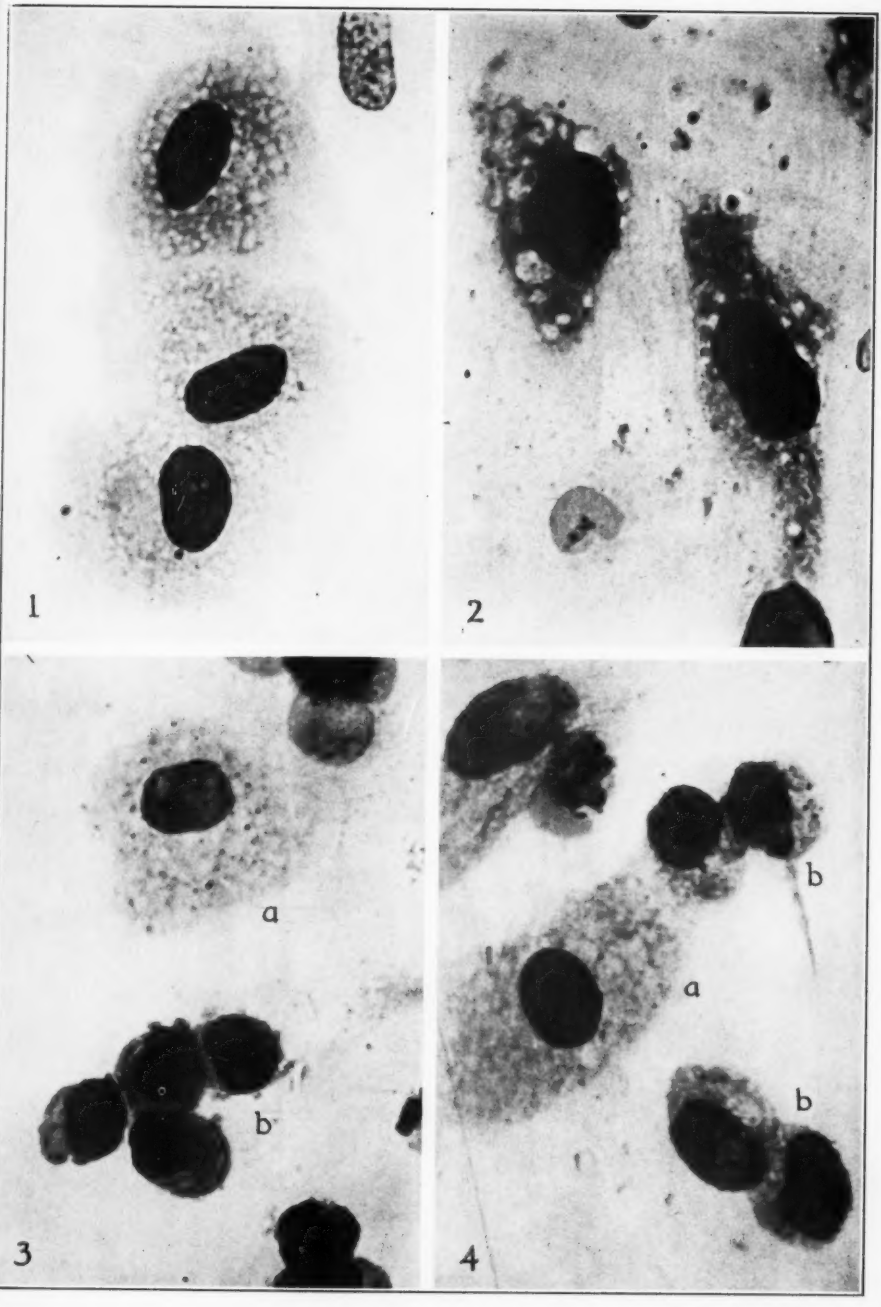

1
2
a
b
3
b
a
b
4

PLATE 72

FIG. 5. a = Histogenous macrophages with metachromatic granulation; b = hematogenous macrophages (hypertrophied lymphocytes). Nuclear similarity of macrophages is now evident. c = Fibroblast undergoing mitotic division at 26 hours. × 1100.

FIG. 6. a = Histogenous macrophages; b = hematogenous macrophages at 49 hours. × 1100.

FIG. 7. a = Histogenous macrophages; b = hematogenous macrophages; c = fibroblast. Note similarity of macrophages at 76 hours. × 1100.

FIG. 8. Illustrating detail of lymphocyte-hematogenous macrophage transformation at 14 hours. a = Lymphocytes; b = early stage showing less compact nuclear structure. × 1500.

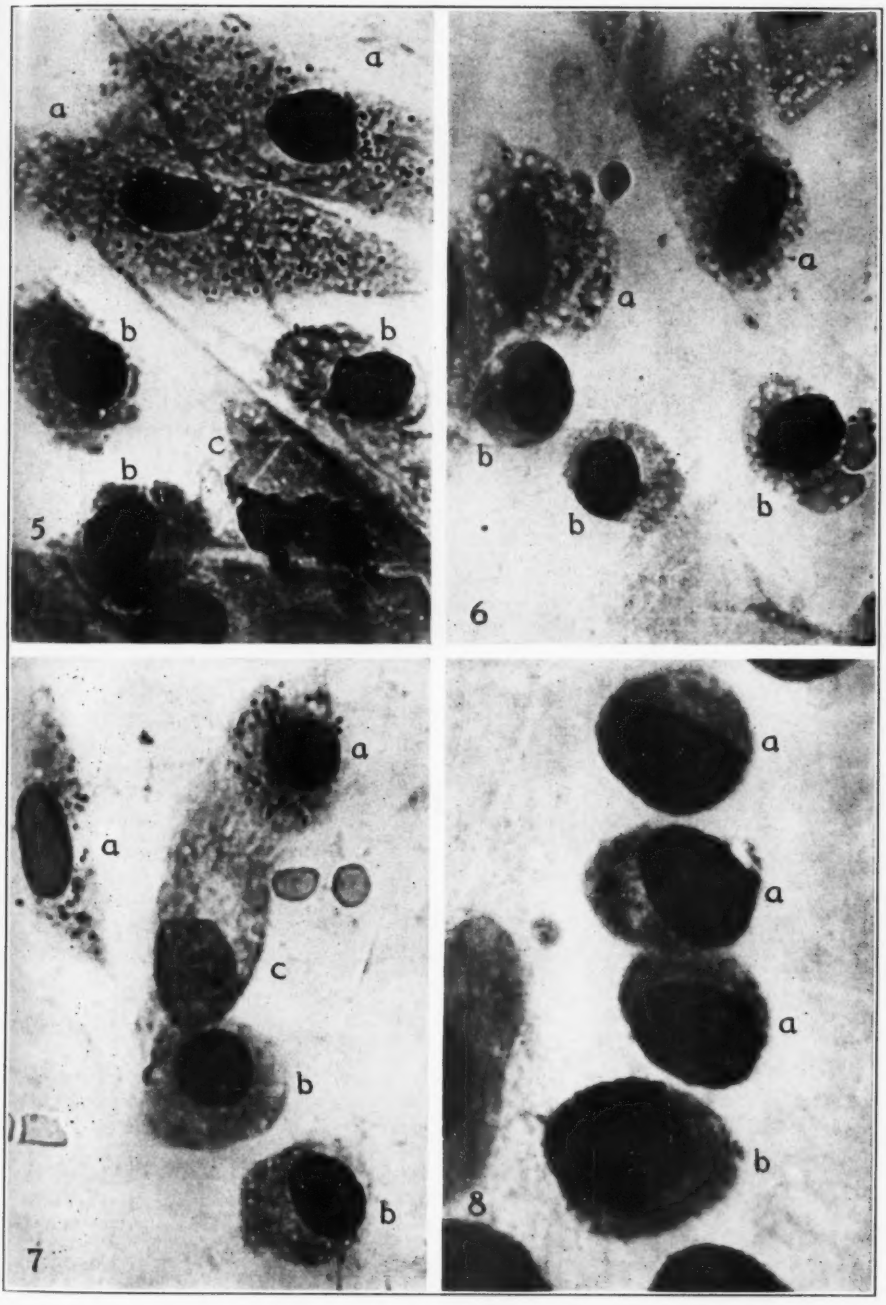

a
a
b
b
c
b
5
a
a
b
b
b
6
a
a
c
b
b
7
a
a
a
b
8

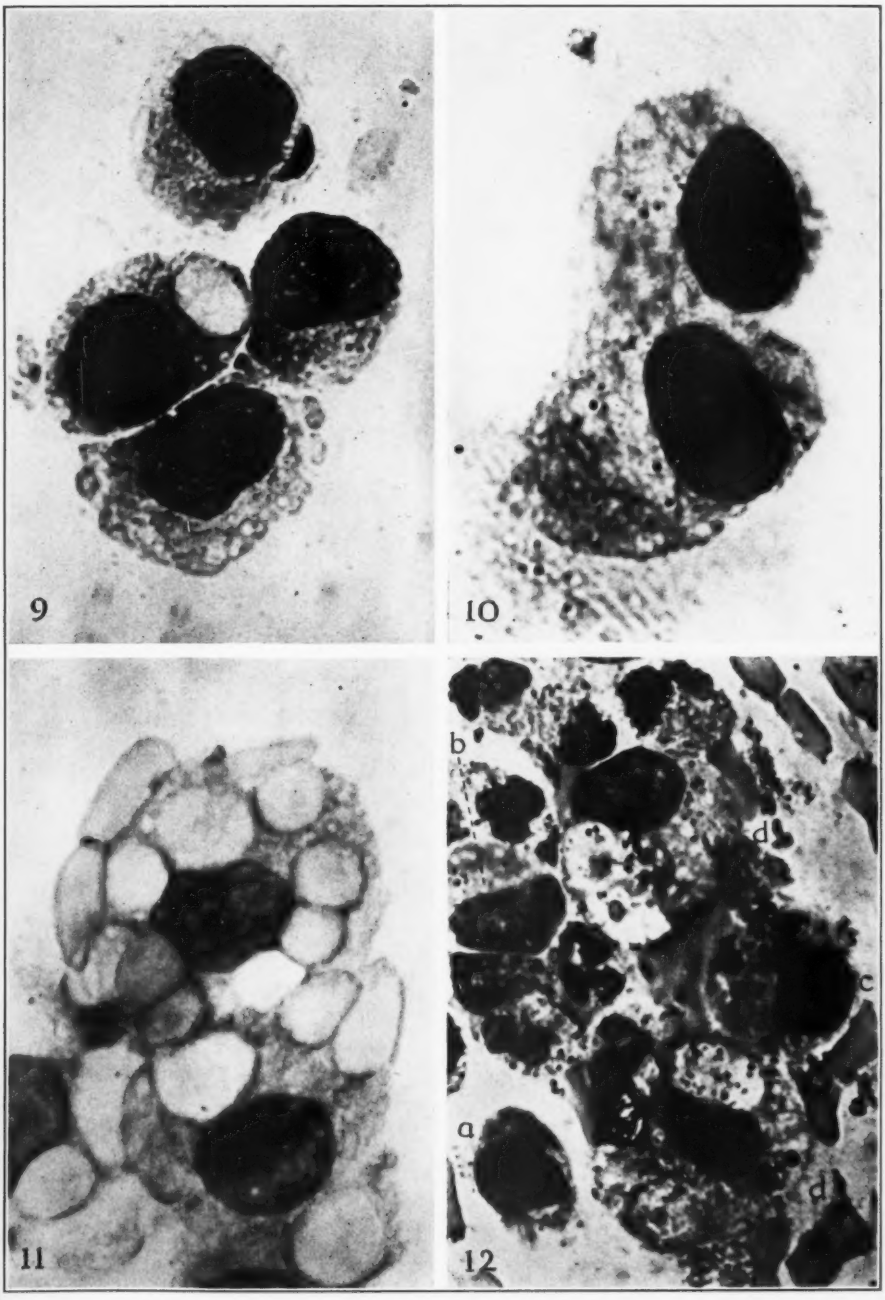
PLATE 73

FIG. 9. Hematogenous macrophages at 18 hours showing an increase in size, a decreased cytoplasmic basophilia and less compact nuclei than the lymphocytes from which they are derived. × 1500.

FIG. 10. Hematogenous macrophages at 26 hours containing a few metachromatic granules. Maximal nuclear hypertrophy is now present but through phagocytosis the cytoplasmic volume may be further increased. At this time the lymphocytic ancestry of the cells is not apparent. × 1500.

FIG. 11. Erythrocytic phagocytosis by hematogenous macrophages. × 1500.

FIG. 12. a, b, c and d show correlation between cytoplasmic volume and phagocytic activity of hematogenous macrophages 38 hours following subcutaneous injection of starch. × 1500.


9
10
11
b
d
c
a
d
12

PLASMA PROTEIN, BILE SALT AND CHOLESTEROL METABOLISM AS INFLUENCED BY MULTIPLE INJECTIONS OF GUM ACACIA IN BILE FISTULA DOGS *

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INTRODUCTION

The liver [1] is the site of production of the bile salts and is the organ normally responsible for the elimination of bile pigments. It has been shown [2] repeatedly that such small amounts of chloroform as 3 cc. given by stomach tube on 3 or 4 successive days will cause a marked decrease in the amount of bile salt excreted by a bile fistula dog. The injury to the liver is slight and there is prompt repair with normal production of bile salts. In some of the dogs after slight chloroform injury there were only traces of bile salt and yet the volume of bile and the amount of bile pigments were little, if any, changed. Whether or not there will be a decrease in volume and bile pigments depends on the severity of the chloroform injury. Chloroform given as an anesthetic for 15 to 30 minutes causes a similar depression in the bile salts output. Injury to the liver effected by phosphorus (injected subcutaneously in olive oil) causes a similar reaction. When [3] the bile fistula is combined with the Eck fistula there results a decreased production in bile salt but the volume and amount of bile pigment in the bile are not affected. This lowered output of bile salt is undoubtedly related to the fact that the liver of the Eck fistula dog is atrophic and its function abnormal.

When [4, 5] there is disturbance in liver function due to obstruction of the biliary tree or to parenchymatous injury, there is an alteration in the cholesterol of the blood. In the case of obstruction the total blood cholesterol becomes elevated but the esters of cholesterol do not increase in a parallel manner. With chronic chloroform injury produced by feeding the drug over an extended period, the total blood cholesterol decreases and the esters of

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We are indebted to Eli Lilly and Company for valuable materials used in these experiments.

cholesterol may diminish actually to the vanishing point. Infection within the liver causes a similar reduction. It is apparent that we have some knowledge as to what may occur when there is disturbed physiology of the liver.

Andersch and Gibson [6] demonstrated that acacia when injected intravenously into dogs is removed and retained in large part in the liver cells. They reported that the bile salt production and bile pigment elimination were reduced. Heckel and co-workers [7] have recently shown that following repeated injections of acacia into the blood stream there results a marked decrease in the plasma proteins with profound reduction in the *plasma fibrinogen* levels. The decrease in fibrinogen from the normal of 300–350 mg. per cent to 50–75 mg. per cent is suggestive evidence that the acacia within the liver cells may be impairing their function at least as regards the production of fibrinogen.

In view of this fact, it was thought that possibly other activities of the liver might be deranged, particularly the bile salt and cholesterol metabolism.

The 2 bile fistula dogs used in these experiments received 4 and 6 doses of acacia by venous injection in 30 gm. amounts. Autopsy revealed that the livers were enlarged, had a glassy appearance with conspicuous lobules, and histologically the liver cells had the peculiar pale reticulated and vacuolated appearance associated with acacia deposition. The bile salt production, bile pigment and bile cholesterol elimination, and the blood cholesterol and plasma proteins have been followed. The plasma proteins decreased and on the days that followed the injection of the acacia there was noted constantly a temporary decrease in the bile salts, but otherwise there has been no effect produced as the result of the liver cells being filled with acacia. The dogs [8] handled the bile salts, when fed, in the normal manner, that is, the salts were absorbed and promptly excreted in the bile. Chloroform and carbon tetrachloride, when fed, caused the expected decrease in bile salt formation.

These data indicate that the acacia within the liver cells causes no serious impairment of their ability to form bile salts. Other experiments [7] point to the possibility that the acacia may interfere with the production of fibrinogen by the liver cells. Such dissociation of functions of the liver is not uncommon.

Methods

The closed sterile bag type fistula as devised by Rous and McMaster[9] and modified by Smith, Groth and Whipple[10] was used. The dogs were fed the salmon bread diet since it is one that is low in fat and rich in carbohydrates and therefore suitable for a dog totally deprived of bile. Its preparation has been described in detail.[11]

Bile salt determinations were made following the method[12] of Foster and Hooper and entail the estimation of the amino nitrogen as determined by the method of Van Slyke.

Methods for bile pigment,[13] bile cholesterol,[14] total blood cholesterol, and esters[15] determinations may be found in detail in previous papers.

Plasma proteins were determined by the macro-Kjeldahl method, using selenous acid as the oxidizing agent.

Hemoglobin was determined following the method described by Robscheit.[16] Standard is equivalent to 13.8 gm. of hemoglobin per 100 cc.

The gum acacia was furnished by the Eli Lilly Company in ampoules containing 30 gm. of acacia in 100 cc. of solution. The contents of an ampoule were mixed in 150 cc. of Locke's solution and injected intravenously from a gravity bottle.

Experimental

Dog 36–9 was a mongrel female and was studied for a period of 123 days after the bile fistula was established. No bile was fed except during the periods indicated in the tables. After an adequate base line period, 30 gm. of acacia in Locke's solution were injected intravenously and determinations made for the following 27 days and then the same sized dose was repeated at intervals of 18, 6, 9 and 22 days. The dog after each injection of acacia would immediately vomit a small amount of mucoid material but within 5 minutes would be quite normal with no further disturbance. Periods of interest are given in the tables.

Three uncomplicated periods are illustrated in Table I. On the day after the 2nd injection of acacia there is a decrease in the bile salt production. The bile cholesterol is slightly lower on this day also and this may be related to the lessened production of bile salt. Wright and Whipple[17] have shown that the cholesterol in the bile

TABLE I

Repeated Intravenous Injections of Acacia

Dog 36–9

Date	Bile volume	Bile pigments	Bile salts	Bile cholesterol	Total blood cholesterol	Blood cholesterol esters	Blood cholesterol esters	Plasma proteins	Hemoglobin	Weight
	cc.	*mg.*	*mg.*	*mg.*	*mg./%*	*mg./%*	*%*	*gm./%*	*%*	*kg.*
Nov. 24	172	83	2183	13.2	71	48	67	6.01	117	16.1
Second Injection of Acacia (30 gm.) on November 24th										
25	124	82	1449	9.8	73	48	66	4.92	...	...
26	164	99	2275	14.1	72	49	68	5.01	...	...
27	132	100	2000	14.8	59	42	71	4.99	...	16.1
28	156	87	1541	...	..	..	..	...	...	...
29	140	86	2073	17.3	56	39	70	5.12	117	16.2
Third Injection of Acacia (30 gm.) on December 12th										
Dec. 12	197	92	2036	...	..	..	..	5.54	...	...
13	180	106	1651	14.2	70	48	69	4.94	...	16.1
14	162	152	1780	13.2	52	41	79	4.48	102	...
15	182	151	2051	11.3	51	32	63	4.73	...	16.1
16	154	123	1780	14.7	56	35	63	4.66	...	...
17	190	125	2220	...	49	36	74	4.93	...	16.1
Fifth Injection of Acacia (30 gm.) on January 18th										
Jan. 18	206	79	2110	25.4	47	30	64	5.38	109	16.1
19	180	98	1651	11.2	52	32	62	4.25	...	...
20	180	121	1853	22.2	42	..	..	4.26	...	...
21	208	116	2550	19.6	42	29	69	4.62	...	...
22	232	98	2624	26.9	51	32	63	4.76	...	16.2

30 gm. acacia injected on October 28th, November 24th, December 12th, 18th and 27th, and January 18th. Total 180 gm.

fluctuates with variation in the bile salt content. Bile pigments and blood cholesterol levels are not altered but there is definite decrease in the percentage of circulating plasma proteins with return to normal levels by the 5th day after the injection. Normal values were obtained for the days following this period and so on December 12th a 3rd injection was given. The bile salt output on the following 2 days was again decreased but returned to normal levels. Blood and bile cholesterol values were not altered. The blood cholesterol, both total and esters, is now lower than in the fore period but the ester percentage remains the same. This lowering of blood cholesterol with maintenance of relation of total to esters of cholesterol is a phenomenon that always occurs in fistula dogs after a period of total bile deprivation. The plasma protein percentage dropped and on the 8th day was 4.25 and remained at this lower level and only after 21 days was there a return to a low normal level of 5.07 gm. per cent. The bile pigments were elevated after the injection. This is not a constant finding and possibly is related to a slight amount of red cell destruction. After the 5th injection of acacia there is again slight excess of bile pigment, some decrease in bile salt with immediate recovery, and an even higher level than on previous days. Bile cholesterol decreases on the day of low bile salt output, but the blood cholesterol shows no change of significance. The blood proteins decrease. It is important to note that the dog's weight has remained constant. The hemoglobin percentage is slightly lowered, but 15 cc. samples of blood were being removed daily. From these data it is apparent that the presence of the acacia in the liver has not interfered with the orderly elimination of bile pigment and bile cholesterol, or the production of bile salts. The fact that the blood cholesterol levels are normal indicates also that the acacia is causing no profound injury to the liver cells.

The effects of obstruction, chloroform feeding and bile salt feeding are clearly brought out in Table II. After the 4th injection of acacia the fistula tract became totally obstructed. On 3 days there were 50, 30 and 30 cc. of pale, watery green fluid and no determinations were performed on this so-called bile. The total blood cholesterol shows the effect of the obstruction as the total cholesterol increases markedly and the ratio of total to esters is slightly lowered. This is the expected reaction to obstruction. On

TABLE II

Effects of Obstruction, Bile Salt and Chloroform Feeding on the Acacia Filled Liver

Dog 36–9

Date	Bile volume	Bile pigments	Bile salts	Bile cholesterol	Total blood cholesterol	Blood cholesterol esters	Blood cholesterol esters	Plasma proteins	Hemoglobin	Weight
	cc.	*mg.*	*mg.*	*mg.*	*mg./%*	*mg./%*	*%*	*gm./%*	*%*	*kg.*
Dec. 27	214	79	2330	6.9	45	34	76	...	..	16.2
Fourth Injection of Acacia (30 gm.) on December 27th										
28	150	97	1431	4.9	45	24	53	4.11	..	...
29*	50	40	550	...	60	36	60	4.29	..	16.2
30*	0	..	....	...	74	42	57	4.21	99	...
31*	30	16	....	...	119	67	56	4.73	..	16
Jan. 1	0	..	....	...	151	79	52	...	..	...
2	0	..	....	...	..	..	..	...	..	...
3*	30	..	....	...	..	..	..	...	..	...
4	320	148	1743	...	..	..	..	...	..	...
5	320	181	2752	17.3	38	22	58	4.83	..	...
6	290	150	2404	21.2	34	22	65	4.89	..	16.2
Bile by Mouth for 3 Days (January 14th, 15th and 16th)										
13	216	78	2257	26.0	43	27	63	5.46	..	16.1
14	260	75	3340	19.3	43	29	67	5.22	..	...
15	236	70	3486	29.2	43	27	63	5.26	..	16.1
16	255	79	3670	...	..	..	..	...	..	...
17	188	66	1982	22.9	50	37	74	5.63	109	16
Chloroform (3 cc.) by Mouth for 3 Days (January 26th, 27th and 28th)										
26	278	85	2624	...	51	32	63	5.09	..	16.1
27	252	89	2257	24.2	42	27	64	4.90	..	...
28	230	81	1046	11.4	49	31	63	5.00	..	...
29	204	79	991	12.0	58	34	59	4.80	..	16.1
30	282	101	1266	...	..	..	..	...	..	...
31	184	50	1101	11.0	66	35	53	5.12	..	...
Feb. 1	296	97	2642	20.4	58	39	67	5.35	..	...

* Dog bile (75 cc.) by stomach tube.

the 4 days indicated in the table 75 cc. of whole bile were given by stomach tube in an attempt to cause an increased flow of bile. The rubber tubing was injected with sterile saline and suction applied also. Between the two procedures successful clearing of the fistula was accomplished as on the 7th day following obstruction a large volume of normal appearing bile was excreted. There was prompt recovery of the bile salt and bile cholesterol output and the blood cholesterol levels fell to the previous base line. Bile pigments were of course elevated. The value of bile feeding to cause an increase in bile volume and so aid in flushing out the fistula tract is well illustrated by these data. The obstruction may have been partially caused by acacia as it is known to be eliminated in the bile.

In the normal fistula dog bile salts, when fed, are absorbed and quantitatively excreted promptly in the bile. In order to see whether the liver cells packed with acacia would function in a similar manner, whole bile (100 cc.) was given by stomach tube on 3 successive days. These portions of bile contained 1006 mg., 1045 mg. and 1478 mg. of bile salt, respectively, or a total of 3529 mg. During a 10 day control period the daily output of bile salt averaged 2161 mg. and as the result of the bile feeding there was an increased elimination of 4013 mg. of bile salt so that there was complete excretion of the 3529 gm. fed. The bile cholesterol increased also, due to the increased amount of bile salts in the bile. Bile pigments and blood cholesterol are not affected. Again there is a normal reaction in spite of the acacia in the liver cells.

When chloroform (3 cc.) is given by mouth to the dog while its liver is still filled with acacia, we see that bile volume and pigments are not affected in the slightest but there is a successive decrease in the bile salt and bile cholesterol output with return to normal on the 4th day after the drug was stopped. Blood cholesterol is not affected as the injury to the liver by this small amount of chloroform is minimal and acute in character.

A week following the chloroform feeding experiment the fistula tract became obstructed and after 4 days the dog was killed with ether anesthesia followed by immediate autopsy. There was jaundice of the sclerae and mucous membranes. The serous cavities were normal except for some adhesions about the rubber tubing in the peritoneal cavity. The heart and lungs were normal

grossly and histologically. Blood clots were normal in appearance and consistence. The spleen was firm and red in color, with the malpighian bodies standing out distinctly as glistening gray nodules 1 mm. in diameter. Sections showed less numerous red cells and pulp cells but scattered throughout there were large phagocytic cells that were pale staining, frothy and vacuolated. These cells apparently had taken up some of the acacia.

The liver weighed 900 gm. and its capsule was smooth. Normally the liver of a dog of this size would weigh about 350 gm. Cut surfaces had a light yellowish brown cast due to staining with bile pigment. The lobules were large and regular and the gland had a glassy appearance due to the acacia. It was friable in consistence. The extrahepatic ducts were markedly dilated and the common bile duct measured 1.5 cm. in circumference. It contained whitish fluid with some flecks of green colored material. The cannula was in place within the duct but it was obstructed by dry, greenish inspissated bile.

Histological sections show inspissated pigment within the small bile canaliculi. The architecture is normal but the liver cells are large and they appear frothy, vacuolated or reticulated with no normal appearing cytoplasm. This abnormal appearance is due to the contained acacia. Sections of the liver when stained with sudan IV are found to contain no fat in the liver cells. The gastrointestinal tract, pancreas, adrenals, kidneys, ureters, bladder, uterus, tubes and ovaries are all normal. The bone marrow shows the normal distribution of marrow.

Dog 37–106 was a female mongrel Airedale and was followed for 84 days after the bile fistula was established. It received no bile except as indicated in the tables. After an adequate base line period, 30 gm. of acacia were injected intravenously with repeated injection at intervals of 8, 6 and 12 days. This dog also vomited immediately after each injection but recovered promptly with no further disturbance.

The two periods illustrated in Table III are typical of the reaction following each injection of acacia. After the 1st injection there is a slight decrease in the bile salts excreted on the day following the injection. The bile pigments and bile cholesterol are not affected nor is there any alteration in the blood cholesterol levels. However, there was prompt and marked decrease in the

TABLE III

Repeated Intravenous Injections of Acacia

Dog 37–106

Date	Bile volume	Bile pigments	Bile salts	Bile cholesterol	Total blood cholesterol	Blood cholesterol esters	Blood cholesterol esters	Plasma proteins	Hemoglobin	Weight
	cc.	*mg.*	*mg.*	*mg.*	*mg./%*	*mg./%*	*%*	*gm./%*	*%*	*kg.*
Mar. 15	114	55	1908	20.0	92	65	71	5.00	116	...
First Injection of Acacia (30 gm.) March 15th										
16	70	53	1431	15.6	79	54	68	3.50	...	15.3
17	82	73	1798	16.7	75	52	70	3.56	...	...
18	80	15	2037	15.4	65	45	69	3.69	...	...
19	84	56	1982	17.5	73	52	71	3.75	...	...
Third Injection of Acacia (20 gm.) March 29th										
29	122	53	1982	16.7	50	36	72	3.31	120	...
30	90	55	1706	15.3	49	37	76	2.42	...	...
31	92	55	1945	17.7	46	32	70	2.63	...	15.6
Apr. 1	100	59	2092	17.9	36	25	70	2.38	...	...
2	102	51	2055	19.2	34	24	71	2.56	...	...

30 gm. acacia injected on March 15th, 23rd and 29th, and April 10th. Total 120 gm.

percentage of circulating plasma proteins and this lower level was maintained. After the 3rd injection there is again a slight decrease in the bile salt excreted on the following day and there is further reduction in the plasma protein. It is of interest that no edema ever developed, even though the plasma protein level was much below 4 per cent. The acacia that remained in the blood compensated for the reduced plasma proteins and prevented formation of edema.

The total blood cholesterol is now at a lower level than previously but the ratio of esters of cholesterol to total is maintained at the normal level of 70 per cent. This is the effect of total deprivation of bile as mentioned previously.

In Table IV the results of feeding bile salts are tabulated. Four samples of dog bile (75 cc.) were given by stomach tube on succeeding days. These portions of bile contained 1219 mg., 1402 mg., 1456 mg. and 1454 mg. of bile salt, respectively, or a total of 5531 mg. Over a 10 day control period the bile salt excretion averaged 2027 mg. daily and during the experimental period 4773 mg. were excreted in excess, or 86 per cent recovery of the amount fed. The bile cholesterol is increased also as the result of the extra salt in the bile, but the blood cholesterol is not altered. The plasma protein continues at the low level consequent to the previous acacia injections.

The dog was next starved for 1 day and then chloroform (5 cc.) was given by stomach tube on the following 2 days. There is immediate decrease in the bile volume with the bile salt content reduced and it is only after 6 days that the bile salts return to normal. The bile cholesterol is reduced in amount but the blood cholesterol is not affected, and the bile volume returns to normal with increase in the amount of bile pigment eliminated. The plasma proteins still are low. Ten days after this period carbon tetrachloride (5 cc.) was given by stomach tube. The bile salts decreased from 1541 mg. to 917 mg. following this 1st dose. The dog was less active on this day but did not appear ill and another 5 cc. dose was given. The next morning the dog was found dead as the result of severe hemorrhage into the peritoneal cavity. The plasma proteins were at the low level of 2.9 gm. per cent and the fibrinogen would be greatly reduced, as illustrated by data on similar dogs.[7] Another factor that may well be responsible for the

TABLE IV

Effects of Bile Salt and Chloroform Feeding on the Acacia Filled Liver

Dog 37–106

Date	Bile volume	Bile pigments	Bile salts	Bile cholesterol	Total blood cholesterol	Blood cholesterol esters	Blood cholesterol esters	Plasma proteins	Hemoglobin	Weight
	cc.	*mg.*	*mg.*	*mg.*	*mg./%*	*mg./%*	*%*	*gm./%*	*%*	*kg.*
Bile by Mouth (75 cc.) April 4th, 5th, 6th and 7th										
Apr. 4	110	53	1885	19.6	40	25	63	2.69	...	...
5	170	66	2844	23.9	40	28	70	2.75	...	...
6	184	63	3174	23.6	44	31	70	2.94	114	...
7	212	63	3633	26.2	48	33	69	3.00	...	15.7
8	198	71	3230	26.0	46	32	70	3.06	...	...
9	150	59	2183	17.6	44	26	59	3.12	...	...
Chloroform (5 cc.) by Mouth April 13th and 14th										
12	70	64	1559	11.1	35	25	71	2.23	90	15.7
13	70	95	1541	14.0	38	28	74	2.44	...	...
14	34	48	734	5.0	35	23	66	2.19	...	...
15	5	..	624	...	35	19	54	2.25	...	...
16	24	61	404	2.0	39	25	64	2.06	...	...
17	60	66	624	...	..	..	..	...	...	...
18	44	94	697	3.9	37	23	62	2.75	...	...
19	70	110	1028	7.1	30	21	70	2.75	101	...
20	94	120	1358	11.1	28	20	71	2.81	...	15.5

spontaneous hemorrhage is the low prothrombin resulting from lack of bile in the intestine. Chloroform and carbon tetrachloride causing injury to the liver may in themselves lower the fibrinogen and prothrombin, so it is not surprising that spontaneous hemorrhage occurred.

At autopsy the peritoneal cavity was filled with unclotted blood but no definite bleeding point could be established. The pleural and pericardial sacs were normal. The heart and lungs were normal grossly and histologically. The blood within the heart was not clotted and no clot formed on standing. The liver was nearly twice the normal size and was friable and reddish in color. Its lobules were indistinct. The cannula was *in situ* in the common bile duct and the ducts all appeared normal. The gastro-intestinal tract contained some granular blood stained material in the terminal ileum but no bleeding points were found. Other organs appeared normal.

Histologically the liver shows extreme necrosis as only a few viable cells remain about the portal areas. These cells are vacuolated and reticulated in appearance due to the acacia. The rest of the lobule shows hyaline necrosis with cells indistinct and with no nuclei. Red cells are numerous in the necrotic areas. Sections of liver stained with sudan IV show no visible fat present.

Discussion

It is apparent that in bile fistula dogs the plasma proteins are markedly reduced in amount following repeated intravenous injections of acacia. The livers of such dogs are increased in size due to the accumulation of the acacia within the liver cells. Heckel and co-workers [7] have shown that with the decrease in plasma proteins there is particularly a concomitant much greater reduction in the fibrinogen. It is to be admitted that the plasma proteins may be decreased to compensate for the acacia which continues to circulate in the blood stream. However, the fact that the reduction in fibrinogen is marked and not in proportion with the decrease in total plasma proteins suggests strongly the possibility that the acacia within the liver cells is interfering with their function of producing fibrinogen. The data obtained from these fistula dogs indicate that the presence of the acacia does not interfere with the activity of the cells in forming bile salts or in eliminating bile pig-

ments. Furthermore, the normal relation between the total cholesterol and esters of cholesterol in the blood is maintained and this might well be expected to be altered if the acacia were causing any serious injury to the cells. The total cholesterol does decrease but the ester percentage remains in the normal range. This is the result of the total bile deprivation causing disturbances in absorption of fatty materials from the intestinal tract. When bile salts are fed they are absorbed and the liver cells immediately excrete them into the bile, and from our data it appears that acacia does not interfere in this enterohepatic cycle of bile salt metabolism.

Andersch and Gibson [6] previously reported that the bile salt and bile pigments were reduced in amount following the intravenous injection of acacia. They describe a bile that loses its normal pigmented color. This is similar in character to the fluid that was excreted by one of our dogs when the fistula became obstructed. We have repeatedly observed such bile in other fistula dogs whose fistula has become obstructed or infected. In view of these facts we feel their data regarding bile salts and bile pigment are not significant but the other data relating to the removal of acacia from the blood stream by the liver cells and its elimination in the bile are correct.

One constant finding in our experiments is that on the day following the injection of the acacia there was a decrease in the bile salts excreted. There is no prolonged reduction and therefore it does not seem that this decrease is due to the presence of the acacia in the liver cells. It is possible, however, that immediately following the injection the functions of the liver cells are temporarily slightly deranged due to the intrusion of the acacia. Another factor in this 1 day decrease in bile salt excretion may be the vomiting that occurred immediately following the injection of the acacia. In other fistula dogs we have observed that vomiting may affect the amount of bile salt excreted.

Since fibrinogen production is affected by the acacia in the liver cells without impairment of their ability to form bile salts, we have still another instance of dissociation of the functions of the liver cells. It might be well to mention a few other examples of such dissociation. A mild liver injury may greatly reduce the bile salt production but the volume of bile and the amount of bile pigments eliminated may remain normal. Likewise, bile salts [2] and pro-

thrombin [18] may be low but the fibrinogen remains normal when injury is not severe. In bile fistula dogs [19, 20] the fibrinogen may be normal and yet the prothrombin may be so decreased in amount that spontaneous bleeding occurs. Inflammation [21] anywhere in the body may cause great elevation in the fibrinogen with no change in the prothrombin. Since the liver has such a wide variety of functions and since there is very frequently this dissociation, it is not surprising that no adequate test of liver function has been established.

Summary

The repeated intravenous injection of acacia in bile fistula dogs results in enlargement of the liver due to the accumulation of acacia in the liver cells. The plasma proteins progressively decrease and are maintained at a level much below normal as a result of the acacia within the blood stream and the liver.

Acacia within the liver cells does not seriously interfere with the cell's ability to form bile salts or eliminate bile pigments, and it does not disturb the enterohepatic cycle of bile salt metabolism when bile salts are fed. The fed bile salt is absorbed and excreted promptly into the bile.

Bile and blood cholesterol metabolism are not altered as the relation between the total blood cholesterol and esters of cholesterol is maintained within the normal range. In the bile fistula dog the total cholesterol of the blood decreases, but this is related to faulty absorption of fats due to total bile deprivation.

Chloroform and carbon tetrachloride when given by mouth cause injury to the liver with reduction in bile salt formation. The injury in one instance was severe enough to cause spontaneous bleeding with death from hemorrhage, indicating interference with formation of fibrinogen and prothrombin.

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MOOSE ENCEPHALITIS *

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A disease of obscure nature affecting moose was referred to in the literature by Cameron and Fulton [1] in 1926, but practically all that is definitely known about the condition has been contributed by Fenstermacher and Jellison,[2] Fenstermacher,[3, 4] and by Thomas and Cahn and coworkers.[5–8]

The diseased animals when first observed show but little of their customary fear of man. They may be fairly readily approached and may sometimes even be led into captivity. Weakness and unsteadiness, with a tendency to staggering gait but without true paralysis, are common. Less frequently there may be signs of impaired vision or peculiar attitudes of the head. Emaciation is observed in the majority of animals but this is by no means constant.

The condition does not show any definite seasonal incidence. Of 23 cases reported from Minnesota [2, 3, 4] the distribution by months was as follows: April, 6; March, 4; October, 3; January, February and May, 2 each; June, July, September and December, 1 each. In Maine † the incidence of 20 cases was: March and April, 4 each; February, 3; May and June, 2 each; and January, July, August, October and December, 1 each. The majority of the cases have thus occurred in the months from February to May, but every month except November has had at least 1 case.

In pathological observations (sometimes incomplete) of 23 animals [2, 3, 4] a variety of parasites was observed in the lungs, liver, intestine, heart and the eye in different animals. Most of the animals showed infestation with the tick *Dermacentor albipictus*, frequently to an extremely severe and extensive degree. However, a rare animal may show no ticks at all and others show only minor degrees of infestation. Many of the moose showed a secondary

* An opportunity to study this disease was presented through the cooperation of Prof. E. C. Nelson of the University of Maine, and of Dr. F. Fenstermacher of the University of Minnesota.

† Personal communication from Prof. C. M. Aldous and Mr. A. L. Lamson.

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type of anemia with basophilic stippling in the erythrocytes. In 2 cases evidence of inflammatory reaction was found in the brain. In 1 of these 2 animals an unidentified nematode was also found in the brain but without attending reaction. The inflammation occurring elsewhere in the brain was probably independent of this parasite. In 4 additional brains only small areas of hemorrhage were found. Apart from these observations in the brain, pathological features that might be relevant to the disease picture were not noted.

Attempts at autopsy to recover pathogenic infectious agents were uniformly unsuccessful, not only by ordinary bacteriological culture, but also by intracerebral or other inoculation of tissue suspensions, including that of the brain.[2, 3, 4] On the other hand, Thomas and Cahn and associates [5–8] studied ticks infesting pieces of moose hide cut from dead animals and shipped to Illinois. When guinea pigs were infested with these insects a fatal disease was produced supposedly similar to what had been described in the moose. From the ticks a new bacterium was isolated, *Klebsiella paralytica*, whose properties have been carefully described [8] and which was suggested as the cause of the disease in moose. However, Fenstermacher, working directly with moose as soon as they were killed, has been unable to confirm these observations. A bacterial origin for "moose disease" cannot be considered as established.

A typical example of the disease was observed at the University of Maine, with the following history.*

On October 21, 1938, a moose was reported wandering around on the highways by the wardens near West Rockport, Maine. It seemed fairly strong but was thin and very tame. It had been seen for 3 days when reported. The animal was loaded into a truck and brought to Orono and placed in about an acre sized pen in the woods. In the pen it showed a good appetite and browsed; it was also fed carrots, cod liver oil and bone meal. On November 10th marked drowsiness, drooping of the hind quarters and a tendency to fall developed. On November 13th the animal fell in a brush pile and was able to get up only with help. A peculiar lopping and a flick of the right ear were noticed, as well as an inter-

* Gathered by Mr. Lamson and Dr. Witte, and kindly supplied to the author by Prof. E. C. Nelson.

mittent facial twitch on the right side. On November 14th the animal went down and could not get up even with help. A pronounced right twist of the neck was noted the next day. On November 16th the moose lay flat on its left side with the neck extended. Marked edema of the eyelids was present and the eye on the left side was clouded and apparently blind. The animal was shot through the heart and autopsied immediately.

The head was removed at autopsy, packed in solid carbon dioxide and shipped to this laboratory, where it arrived on the 2nd day following. The tissues were in an excellent state of preservation, considering the lapse of time since death. On removal of the brain no gross abnormalities were observed. The nose, sinuses and ears were entirely normal. The edema of the eyelids mentioned in the history was not observable after the lapse of time involved during shipment, and the eyeballs showed no abnormalities. Some of the brain material was saved for passage and the remainder fixed in 10 per cent formalin or in Zenker's fluid. This animal is designated hereafter as Moose 1.

For further study the following additional material was obtained from Prof. Nelson: portions of a brain and cord of a 2nd moose fixed in 10 per cent formalin for 11 months, and an entire brain of a 3rd animal, fixed in formalin for 8 months. Dr. Fenstermacher kindly furnished paraffin blocks of parts of the brain from 5 additional animals which had been autopsied in Minnesota. Material from 8 animals was thus studied.

Observations

The following description is drawn chiefly from Moose 1 whose history has been detailed above. Reference to the other cases is made where indicated.

In the brain several different types of pathological change may be observed, the interrelations of which it is not always easy to determine. There is a definite loss of myelin, although of an unusual type. In frozen sections stained with scarlet red considerable quantities of neutral fat may be observed in scattered parts of the white matter. In Figure 1 is seen a low power view of a portion of the corpus callosum illustrating an unusually severe degree of fat formation. The fat stains a brick red, rather dull in color, without the brilliance frequently seen in other demyelinizing

conditions. Under polarized light there is no double refraction. The lipoid droplets do not stain with hematoxylin in myelin stains. That all the fat is intracellular, within phagocytes, cannot be satisfactorily shown. Stains of cells in paraffin embedded tissues show very few typical compound granular corpuscles. This may be due to cytoplasmic disintegration in the period between death and fixation of tissue, or it may be due to the absence of such cells. That neutral fat can occur in the brain in the absence of phagocytic or other cellular action is not surprising, for this type of fat formation has previously been demonstrated in multiple sclerosis.[9] Similar, though less intense areas of fat formation have been found in the centrum ovale and in the white matter of the cerebral convolutions. In such regions, as in Figure 1, the lipoid droplets are scattered more or less uniformly in very poorly defined foci. Sharply circumscribed areas showing loss of myelin, such as are seen in multiple sclerosis, do not occur. Where the free fat is scattered in the tissue the myelin sheaths may be moderately diminished in number but are not totally lost.

The presence of diffusely scattered fat is indicative of an acute process. In other portions of the brain there is an accumulation of lipoid only in the perivascular spaces, but none in the intervascular areas. Such a field is illustrated in Figure 2 and is a sign of an older process than that shown in Figure 1. The total amount of fat observed in the entire brain, both in the parenchyma and in the perivascular spaces, is not large.

In other regions, where there is no sign of recent destruction, many scarred areas demonstrable with stains for glial fibrils are found. These scars are present in the medulla, centrum ovale and convolutional white matter of the cerebrum, and also in the white matter of the cerebellum. In Figure 3 is seen such a scar in the medulla. The proliferation of glial fibrils is quite intense but the actual increase in astrocytes, *i.e.* in the number of cell bodies, is not great. The occurrence of intense fiber proliferation, in the absence of significant cellular increase or of progressively altered cell forms, shows that the repair process is completed. The original insult occurred probably some months previously.

Two types of glial scars are illustrated in Figures 4 and 5. Figure 4 is from the centrum ovale of Moose 1 and shows several small perivascular scars. Figure 5, from the cerebellum of Moose

6, shows a dense isomorphous glial feltwork, diffuse rather than perivascular. Here the process is quite old, for well defined astrocytes are rare, although the fiber proliferation is great. On the other hand, in Figure 6, from Moose 1, astroblastic forms are clearly seen among the glial fibers.

The loss of myelin is generally mild, invariably perivascular, and practically always much less in extent than is the fibrous gliosis. In Figure 7, from Moose 1, the destruction of myelin, although slight in absolute terms, is very severe as compared with that in other parts of the brain. In Figure 8 is seen a section, adjacent to that shown in Figure 3, of the medullary reticular formation but stained for myelin. Considering the thinness of the section (12 μ) it is readily seen that the loss of myelin is disproportionately small compared with the density of the gliosis (Fig. 3). And in the myelin stained section adjacent to that of Figure 5 no loss of myelin at all can be discerned in the corresponding area. In the section adjacent to that of Figure 4, but appropriately stained for myelin, there are small clear areas surrounding the affected blood vessels.

Axis cylinders are somewhat better preserved than the myelin sheaths but not to any marked degree. But, as has been emphasized elsewhere,[10] in the loss of myelin from whatever cause the axis cylinders are always less affected than the myelin sheaths.

There is abundant cellular reaction, chiefly perivascular. In Figure 2, for example, the cells in the blood vessel sheaths are chiefly concerned with the phagocytosis of lipoids. Elsewhere, however, as shown in Figure 9, there may be a true inflammatory reaction with abundance of lymphocytes around the blood vessels and even some diffuse tissue infiltration. Polymorphonuclear leukocytes were not seen. This inflammation may be of the "secondary" or symptomatic type for, as is well known, it is frequently seen in various non-infectious demyelinizing diseases. In the sections corresponding to Figure 9, but stained for glia and myelin, a fibrous gliosis could not be demonstrated. Around the more severely involved blood vessels was a mild degree of loss of myelin.

With one single exception, the inflammatory reaction, where present, was restricted to the white matter. In the exceptional instance (Moose 5 of this series) the gray matter was affected.

This occurred in 1 case sent by Dr. Fenstermacher where, slightly involving the entorhinal cortex, there was a reaction very similar to what he has illustrated in his Figure 3.[3] Moose 5 of this series is the same animal from which Dr. Fenstermacher's photograph was taken.

The neocortex was intact in all available material. For the most part the tissue was not sufficiently well preserved for cytological examination. Most of the ganglion cells showed severe swelling, vacuolation, and other postmortem artefacts. But the architectonics appeared normal and no areas of loss of cells, inflammation, or of meningitis were observed.

Of the 8 cases available for study, 3 came from Maine, of which 2 showed similar pathological lesions, while in the 3rd no lesions of any sort could be found. This last case, however, stained poorly. Of the 5 cases, blocks from which were sent from Minnesota by Dr. Fenstermacher, gliosis with more or less demyelination was found in 2. In a 3rd, of which only a few blocks were available, the only pathological change observed was the inflammatory reaction in the gray matter referred to above. In the remaining 2 cases no abnormalities could be detected. In 1 of these 2, however, death was probably due to distomiasis and not to moose encephalitis (personal communication from Dr. Fenstermacher; material to be published). Thus, in 7 probable cases of the disease in question, characteristic changes were observed in 4, while 1 other appeared atypical. In reference to the negative findings it should be pointed out that all sick (or dead) moose, even with fairly similar symptoms, are not necessarily affected by a single disease. Fenstermacher [2] is strongly of the opinion that "the losses of moose that occur in Minnesota are not the result of a single pathogen."

From the whole brain that was received unfixed from Maine, portions were emulsified for animal passage. Sheep, kittens, mice and a pig were inoculated. One sheep died of bacterial meningitis, but a 2nd animal survived without symptoms. All the other injected animals also showed no symptoms. These results agree with Fenstermacher's inability to reproduce the disease by inoculation.

Discussion

The occurrence of neutral fat, perivascular areas of demyelination, gliosis, and moderate inflammatory reaction in the brains of moose raises the question of a possible relation to multiple sclerosis and other demyelinating diseases of man and animals. One chief difference from multiple sclerosis is that in the latter disease the areas showing loss of myelin, although frequently perivascular in early lesions, usually develop to have no relation to blood vessels.[11]

In the moose, the loss of myelin is disproportionately small compared with the extent of the gliosis, somewhat reminiscent of the human cases reported by Müller [12] and by Bodechtel and Guttmann,[13, 14] and not at all similar to multiple sclerosis. It is necessary to agree with their statement that gliosis is not merely a defect filler but may be induced independently. In a previous communication [9] it was pointed out that the gliosis which occurs in multiple sclerosis cannot be considered as merely secondary to the loss of myelin. This statement must be repeated in relation to the disease in moose.

It is well known that in the central nervous system there is no necessary connection between inflammation and loss of myelin. Glial proliferation may occur as a result of an inflammatory reaction in which myelin has not been significantly destroyed. Yet in the disease in moose the histological picture does not suggest a primary inflammatory condition such as is found in many virus diseases. An exception to this statement is Moose 5, referred to above, with a typical primary inflammatory reaction involving the gray matter, a condition which does not fit in with other cases of the series. Although in this single case only a few blocks were available for examination, the evidence is strongly suggestive that this one instance may represent a quite different disease entity. In the 4 other positive cases the changes observed were strictly those of a leukoencephalitis. Moose 5 was not in this category.

For the present, until further data become available, this leukoencephalitis must be considered as a disease entity in moose, with the strong possibility that there may also be another form of encephalitis.

It must be emphasized that the disease process as disclosed by the present study is evidently a subacute or chronic condition.

The glial scars are at least of several months duration, and not improbably even older. At the same time activity of the disease shortly before death is shown by the occurrence of neutral fat. The immediate cause of death, however, as in most neurological conditions, is not apparent.

The etiology of the demyelinizing condition remains obscure. Attempts by Fenstermacher and by ourselves to transmit the condition by tissue inoculation have been negative. This failure is not a cogent argument against an infectious etiology, but the evidence is supported by the fact that none of the many forms of leukoencephalitis has ever been shown to be caused by an infectious agent. That the bacterium *Klebsiella paralytica* is the causative agent must remain questionable until confirmed. Attempted confirmation has not proved successful.[3]

The role of the tick infestation remains equally obscure. The condition of tick paralysis, in animals and man, is well recognized as a naturally occurring disease and has been reproduced experimentally.[15, 16] However, there are no adequate studies of the pathology of this condition, and the clinical course does not suggest a kinship. A toxic factor resulting from the tick infestation cannot be arbitrarily ruled out.

Thrombosis and vascular occlusions as the cause of demyelinizing lesions have been claimed by Putnam.[17] In the present instances evidence of thrombosis was not observed.

There is no evidence throwing satisfactory light on the etiology of this disease. Assuming that the great majority of dead or sick moose observed are suffering from a single disease entity, and considering the total moose population of Maine and Minnesota, the incidence of this disease is high, suggesting either an infection or a common environmental factor such as a dietary deficiency or a toxic substance. Grounds for a decision are not as yet available.

Summary

A subacute or chronic leukoencephalitis occurring naturally in moose is described. The characteristic picture consists of a mild degree of perivascular demyelination, with formation of neutral fat, and with fibrous gliosis disproportionate in extent to the loss of myelin. There may be mild inflammation restricted to the white matter. There is suggestive evidence that a primary inflammatory

reaction involving gray matter and observed in 1 animal out of 8 may represent a separate condition. Attempted animal passage of fresh material from 1 case was unsuccessful. The etiology of this leukoencephalitis is obscure although various possibilities are discussed.

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DESCRIPTION OF PLATES

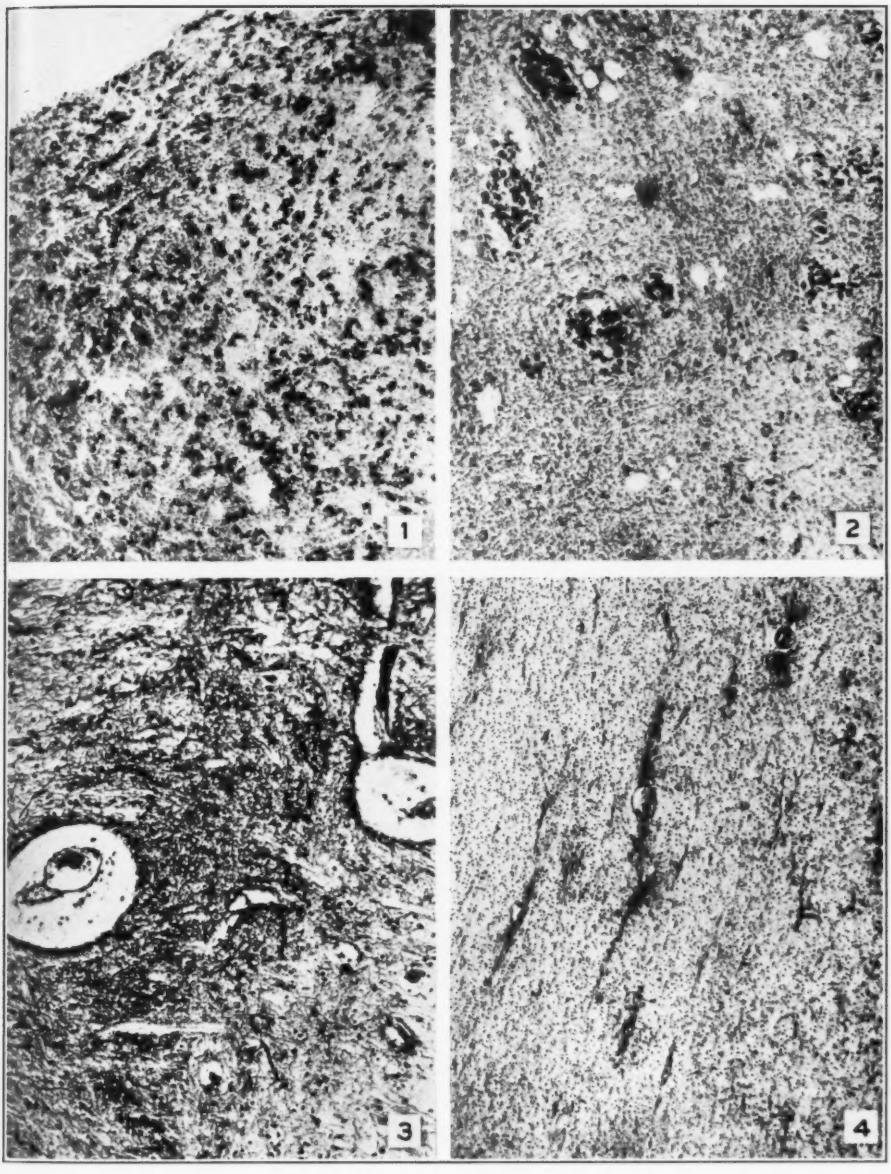
PLATE 74

FIG. 1. Cerebral white matter, stained for fat. The stainable lipoids are diffusely scattered throughout the affected focus. × 50.

FIG. 2. A different field from the same animal. The neutral fat is located almost exclusively in phagocytes in the perivascular spaces. The insignificant loss of myelin can be readily appreciated even with the fat stain. × 133.

FIG. 3. Reticular formation of the medulla oblongata, stained for glial fibers with Victoria blue. Dense, moderately well circumscribed perivascular gliosis is present. This figure should be compared with Fig. 8, the same field of an adjacent section stained for myelin. × 105.

FIG. 4. Centrum ovale, stained for glial fibers. The small scars are strictly perivascular. × 47.5.



King Moose Encephalitis

PLATE 75

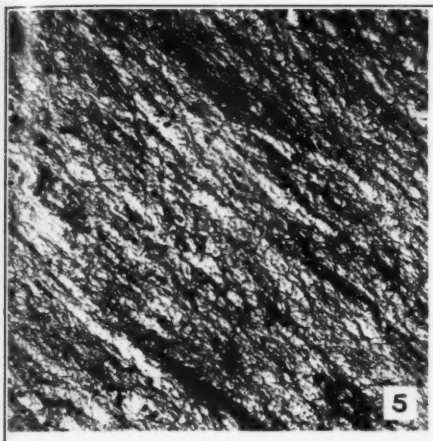
FIG. 5. Cerebellar white matter, stained for glial fibers. There is a very dense glial feltwork. Adjacent sections stained for myelin show no loss of myelin. × 180.

FIG. 6. From the centrum ovale, showing astroblastic forms in the midst of a mild diffuse gliosis. × 230.

FIG. 7. Cerebral white matter, stained for myelin. A slight degree of demyelination is evident around the smaller blood vessels. × 18.6.

FIG. 8. Reticular formation of medulla oblongata, stained for myelin. Fig. 3 shows the dense glial scar in this region. Fig. 8 illustrates the insignificant degree of loss of myelin. × 105.

FIG. 9. Centrum ovale. Inflammatory reaction in the white matter, not invading the cortex, visible at upper left. Toluidine blue stain. × 35.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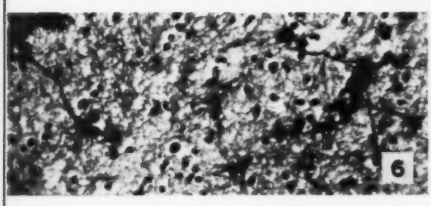
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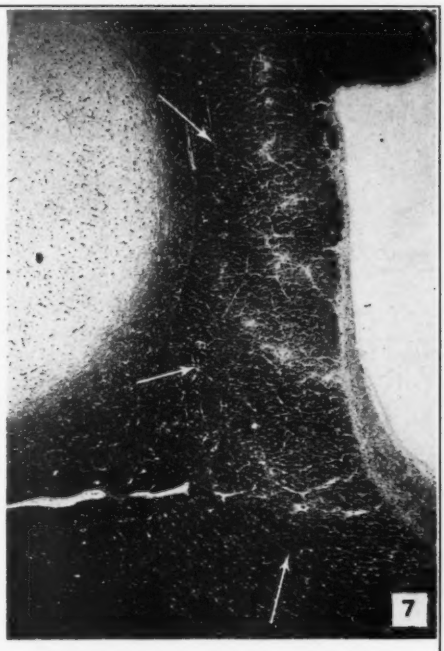
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THE DIFFERENTIATION BETWEEN SPIROCHETES AND SPIROCHETE-LIKE STRUCTURES IN THE PLACENTA *

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INTRODUCTION

The paucity of literature concerning the presence of spirochetes in the human placenta indicates that they have been observed in this location on but few occasions, and furthermore tends to raise the question of the validity of these observations.

Montgomery [1, 2] has expressed the opinion that the discovery of *Treponemata pallida* in the placenta occurs so infrequently as to be of negligible value, and that while the human placenta may transmit the organism of syphilis from an infected mother to the embryo, it seems to possess "a peculiar resistance to the development of true syphilitic lesions" in itself. McCord [3] found no definite histological appearance of the placenta characteristic of syphilis. Routh,[4, 5] in support of an earlier suggestion, states that "certain placental ferments" exhibit treponemicidal activity. Harrison [6] states that spirochetes have not been found frequently in syphilitic placentas but attributes this to the lengthy search required. Kaufmann,[7] however, states: "By the use of Levaditi's silver method, Paaschen, Wallich and Levaditi and many others have found spirochetae particularly in the villi; Mohn, *e.g.*, found them in 50% of the cases in the umbilical cord, and in the placenta, in 70% of the cases of syphilis of the parents."

In a previous paper Dorman and Sahyoun [8] reported the discovery of spirochetes in 105 placentas and outlined the method of examination. The confirmation of these observations by others would contribute materially to the elucidation of the pathogenesis of syphilis, and, for this reason, the full details of the methods used are being included in this paper.

As our methods include silver impregnation, it is worthy of mention that the interpretation of structures so impregnated is prone to be subject to controversy, although there is no more basis

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for general criticism of silver impregnation methods than there is for most staining methods, a view supported by the experimental work of Bofill-Deulofeu,[9] and of Robinow.[10] In the present study, relative to the validity of identification of silver impregnated, spirochete-like structures in placental tissue, we have: (1) applied methods designed to preclude or confirm the alternative possibilities that the observed structures might be fibrinous filaments, collagenous fibers, reticular fibers, mycelia, or other structures which are known to be subject to impregnation with silver after treatment with formalin; and (2) searched for positive evidence that the observed structures are in fact spirochetes.

Material

The placentas examined were chosen by the clinician and were from patients with a positive serum reaction, or with a history of stillbirth, prematurity, or abortion, *i.e.*, from cases that were clinically suspected to be syphilis.[8]

Tissue from a chancre experimentally produced in the scrotum of a rabbit by inoculation with *Treponemata pallida* was used as a control for each of the technical procedures used.

Methods

The fresh placentas were placed in flat jars containing a 10 per cent neutral formalin solution for 24 hours. Ribbons of placental tissue about 0.5 cm. in thickness were obtained by slicing the partially fixed placenta in its longest diameter. Pieces of these ribbons 4 cm. in length were fixed in 5 per cent formalin for a minimum period of 3 weeks. Each piece was then halved by cutting through its most suspicious site and the resulting halves separated into two groups as follows:

Group A: This consisted of one block of each pair which was dehydrated, cleared and embedded in paraffin.

Group B: The analogous block of each pair in this group was impregnated with silver according to Nyka's modification of the Levaditi method,[11] and then dehydrated, cleared and embedded in paraffin.

Six corresponding sections varying in thickness from 5 to 15 μ were cut from each of the twin blocks and each section mounted on a separate slide. Of the 6 sections from each block of Group A,

2 were stained with hematoxylin and eosin, 2 with Weigert's safranelin and hematoxylin, and 2 with Mallory's aniline blue stain.[12] Of the 6 sections from each block of Group B (silver impregnated), 2 were deparaffinized, cleared, and mounted in Canada balsam; 2 were deparaffinized, counterstained with Mallory's aniline blue stain, cleared, and mounted in Canada balsam; and 2 were deparaffinized, washed in distilled water, treated overnight with a 1 per cent solution of potassium iodide, washed gently with bidistilled water, treated with a 5 per cent solution of sodium hyposulphite, washed in distilled water, dehydrated, cleared and mounted in Canada balsam. It was found that the handling of these slides after treatment with potassium iodide had to be done with care as the sections showed a tendency to be displaced easily. The purpose of this procedure was to obtain increased definition of a few of the spirochete-like structures, even though the silver precipitate was removed to such an extent that the number visible was greatly reduced.

Microscopic Examination

Sections from blocks of Group A served the purpose of ascertaining the general architecture of the placenta and of determining the topographical distribution of the pathological lesions. Particular note was taken of hyalinization, infarction, calcification, leukocytic infiltration, and the condition of the blood vessels, recording the changes in the latter as (*a*) no special pathology, (*b*) thickening of the walls with or without occlusion, and (*c*) periarteritis and endarteritis. More than one of these conditions was frequently found in a single section.

Sections from blocks of Group B (silver impregnated) were routinely examined in the following order: (1) ordinary silver impregnated sections; (2) silver impregnated sections counterstained with Mallory's aniline blue stain; and (3) silver impregnated sections treated with potassium iodide.

Observations

A. The ordinary silver impregnated sections are first examined with low power for certain small argentophilic foci. When present, such foci are usually found near the amniotic surface and occasionally on the decidual surface or in the region of the large blood

vessels. These foci are round, oval or serpiginous in shape. In some instances they are large enough to be seen without the aid of a lens. A number of these foci, marked with India ink, are then examined with higher magnifications. Under the oil immersion lens such foci are seen to contain numerous silver impregnated granules and spirochete-like structures. Usually the granules are more numerous in the peripheral zone, and in some instances they are so concentrated in the periphery as to demarcate the focus with a dark ring. The spirochete-like structures are arranged singly or in groups; sometimes two or more are intertwined and appear as a thick cord, or two or three may be intertwined end to end. Study of the above conditions was facilitated by use of a binocular microscope equipped for stereoscopic vision. By this means structures appearing as a tangled mass of black lines can be distinguished from one another as they arise into perspective; their coils are emphasized and the individual filamentous components are optically distinguishable, appearing in their homogeneous background as distinctly discrete, spirally coiled bodies.

B. A counterstain of the silver impregnated sections with Mallory's aniline blue stain was done to differentiate between the collagenous fibers and the spirochete-like structures. Staining with Mallory's aniline blue method following impregnation with silver gives a degree of selective staining analogous to the selective affinities shown by Mallory's stain after Zenker fixation, but not otherwise obtained in formalin-fixed tissues. The staining affinities of the different components of the placental substance from the amniotic surface outward, including the foci, are as follows:

1. The cuboidal cells of the amniotic membrane take a brownish golden color, contrasting with the blue-staining, delicate collagenous fibers which extend from the underlying layer and surround each cell.

2. A thick, deeply stained layer of blue collagenous fibers forms the second layer, from the inner surface of which delicate fibers extend to the amniotic membrane, and from the outer surface of which grow the chorionic villi. This second layer contains embedded in its substance both the large blood vessels and the scattered groups of Langhans' cells, the latter appearing as pale brownish, polyhedral cells with brownish yellow nuclei.

3. The chorionic villi, rich in the blue-staining collagenous fibers of the stroma, are covered by syncytium which is stained purplish violet. Delicate blue collagenous fibers extend from the stroma of the villi and enmesh the syncytial masses.

4. The smooth muscle cells of the blood vessels take a golden brown color and the erythrocytes a golden red.

5. The cells of the decidua take a brownish golden color, conspicuous in their blue environment.

6. Fibrin and fibrinoid filaments, as well as the amorphous hyaline, stain yellowish red.

7. Areas of calcification, when present, appear as amorphous black masses surrounded by a dark background.

8. Infarcted placental substance loses its property of staining in proportion to the age of the infarct. The most advanced infarcts are yellow and contain a few, irregularly distributed, grayish blue collagenous fibers.

9. The foci become yellowish red and appear to be related to the periarteritic and endarteritic blood vessels, as such damaged blood vessels are usually found in the neighborhood and in some instances in the center of these foci. Villi may be found in their vicinity and remnants of villi may even be within the focus, but these structures, owing to their blue collagenous content, stand out prominently in the yellowish red matrix of the focus. The black spirochete-like structures appear as definite forms showing distinct ends which have no connection whatsoever with contiguous blue-staining collagenous fibers or yellowish red, fibrinous filaments. No stage of transition is found between the spirochete-like structures and the blue collagenous fibers. In but few instances are these black structures seen among the blue-stained collagenous masses, but even here they stand out as sharply defined black objects which exhibit no relation to, continuity with, or transition from the blue collagenous fibers.

C. The silver impregnated sections treated with potassium iodide are then examined. The method of clearing the impregnated sections of excess silver ("desilverization," if it may be so called) is a new procedure which has been of material help in our study. The sections become very pale and require painstaking search and thorough study. The areas in which the foci should be found are marked with India ink before examination.

On examination with low magnification very little can be made out as the sections show a uniform, very faint brownish yellow color. With the oil immersion lens it is found that the black granules have lost their color completely and have merged into the background. The majority of the spirochete-like structures appear as almost colorless, transparent refractile filaments, but some have their silver coating sufficiently preserved so they can be sketched with a camera lucida (Text-Fig. 1). The increased sharpness in definition compensates for the decrease in number. The spirochetal structures which have been freed of excess silver appear as cylindrical spiral bodies 10 to 12 μ in length, 1 μ in thickness, and consist of 8 to 10 coils. The distance between the crests of the coils is from 1 to 1.25 μ. The coils are more open toward the ends of the bodies and the ends are tapering. As a result of the treatment with potassium iodide there is a slight increase in the diameter of these structures. This increase, however, amplifies the curvature of the coils and emphasizes their spiral nature.

Study of the syphilitic rabbit tissue which served as a control showed that the appearance of the foci, the location of the spirochetes and their relation to the argentophilic granules, and the staining affinity of the components of the foci were strictly analogous with those features as described in the placenta. Microphotographs would have been duplicates of those of the placenta (Figs. 1–3).

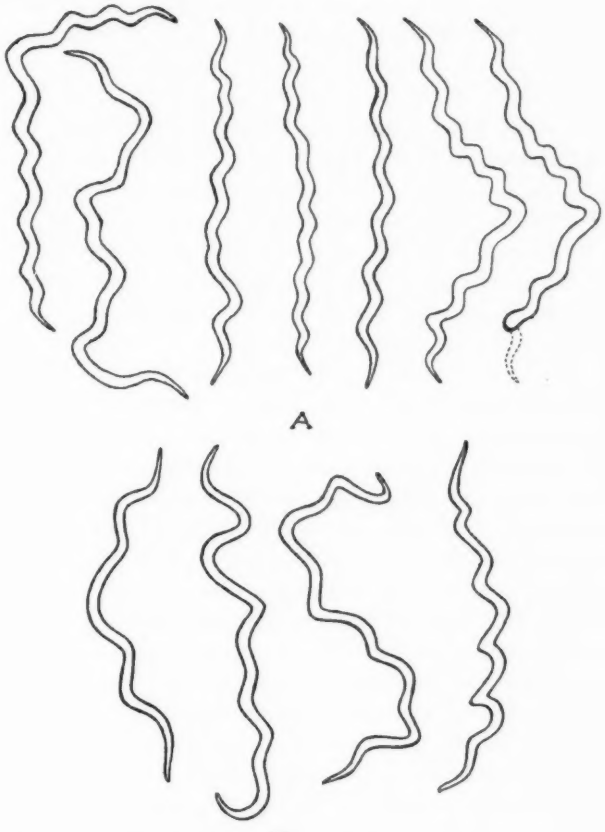
In connection with the above observations and the illustrations, it is important to realize the technical difficulties in the study and illustration of spirochetes in sections. In order to make the nature of these difficulties clear, they may be summed up as follows:

1. Complete spirals as such are not visible under high power, as only those segments of the spirals which happen to be in the plane of focus can be seen at any one time. These appear as interrupted segments of arcs which follow a certain rhythm depending on the size of the coils, their amplitude, and whether the spiral is straight or tortuous.

2. As the focus is changed, that plane of the spiral which was in focus disappears and is replaced by another plane which shows other segments of arcs. These segments follow a rhythm similar

to those in the former plane, but take another direction depending on the plane and the angle of vision.

3. When the spiral is of fine caliber, the segments that can be seen in focus are so small that they appear like beads, and it is, therefore, difficult to show complete spirals in one microphotographic field.



TEXT-FIG. 1. A. Semidiagrammatic camera lucida drawings of 7 spirochetes after removal of excess silver with potassium iodide. In No. 7, one of the ends has been reconstructed, as this end was lying in a plane perpendicular to the field of vision.

B. Semidiagrammatic camera lucida drawings of 4 *Treponemata pallida* from a syphilitic chancre of a rabbit. × 4166.

4. In order to show the complete spiral by means of high power, the observer must focus constantly up and down while drawing. The resulting picture can, at best, be a mental one showing the way the mind of the observer has interpreted the shifting scene before his eyes.

In articles and monographs dealing with spirochetes in sections the value of the statements and microphotographs should not be judged without having in mind the above mentioned optical facts.

Discussion

Study of the hematoxylin and eosin-stained sections serves the purpose of ascertaining the general architecture of the placenta and of determining the topographical distribution of the pathological lesions. Such a study shows that periarteritis and endarteritis are the only findings that suggest the likelihood of finding "spirochetal" foci in the corresponding silver impregnated sections. It is of special interest to note that although the spirochete-like forms are found in the neighborhood, we are unable to find these structures in the occluded vessels themselves. Hyalinization has been seen in all of the placentas we have examined and is of no diagnostic value, even though the foci under discussion usually occur in such areas. Likewise, infarcts have no special significance for our problem as the argentophilic spirals have not been found in them, whether the infarcts be fresh or old. Calcification is so irregular in occurrence and distribution that it must be considered as irrelevant to the problem.

That the foci described in the silver impregnated sections are lesions is strongly suggested by their nature and contents, *i.e.*, they constitute abnormal entities which can be traced through the different sections of the same block, and it is hardly conceivable that accidental defects would be so uniformly consistent as to location, shape and contents.

Homma,[13] who recently studied silver impregnated sections of clinically syphilitic and normal placentas, described certain argentophilic structures found in all of his material, which, because of their size, shape, distribution and lack of relation to anything definable as a lesion, he rightly concluded were non-spirochetal in nature. However, the structures he described have no bearing on the argentophilic spirochete-like structures described in the present paper.

That the argentophilic spirals observed by us are not collagenous fibers is established by the following facts. The described foci, in the Mallory's aniline blue counterstained sections, are of a hyaline appearance, *i.e.*, they take a red stain and are readily distinguished from villi and remnants of villi which may be found in the neighborhood, as these are rich in collagen and as a result stain blue. For the same reason they can be distinguished from blood vessels whether they appear normal, or partially or completely occluded. The foci themselves do not contain collagenous fibers. Examination of these foci with the oil immersion lens shows the spirochete-like structures as definite black objects of uniform shape, clear-cut outline and sharp ends. In contrast, the collagenous fibers are blue and vary in shape, length and caliber; they may be wavy but are never spiralled. These distinguishing features eliminate the possibility of the black spirochete-like objects being collagenous fibers. Moreover, healthy collagenous fibers are never stained uniformly black with silver, and the black spirochete-like structures have not been found in any of the infarcts where degenerating fibers are to be expected.

That the argentophilic spirals are not reticular fibers is established by the following facts. Although the reticular fibers do stain black with silver, they show a definite relation to the collagenous fibers, with transitional stages. Reticular fibers are not uniform in length, thickness or coils. On the other hand, the spirochete-like structures are fairly uniform in shape, size and caliber and they show no relation to, transition from, or continuity with, the collagenous fibers found in the neighborhood of the focus. We considered the possibility of their being fragments of reticular fibers, but after comparing them with known fragments of reticular fibers, and for the reasons cited above, we could find no evidence to support this view.

That the argentophilic spirals are not fibrin or fibrinoid is also established by the following facts. In the counterstained sections the fibrin as seen in the umbilical cord takes an orange-red color, and the widely distributed fibrinoid material, which is so prominent in between the chorionic villi, is similarly stained. We could not determine any transition or continuity between either of these substances and the spirochete-like objects. In connection with the possibility of these structures resulting from the coagulation

of blood, the experimental studies of Stübel,[14, 15] and Barratt,[16] using darkfield illumination, have brought out the fact that in the course of coagulation of plasma the experimental conditions as well as the species of animal supplying the blood determine the character of the structures formed, *i.e.*, the fibers of fibrin may be coarse or fine, or fibrillar structures may not appear at all. Furthermore, Nageotte [17, 18] demonstrated that fibrin is not normally impregnable with silver, but if citrated plasma is mixed with calcium chloride, the fibrils formed in the coagulum can be impregnated with silver. However, the fibrils studied by Nageotte are not constant in size and conformation, as are the structures under discussion.

That the observed argentophilic spirals are not cell boundaries is established by the fact that the so-called "cell-boundaries" are not impregnated with silver after fixation by formalin.

Elastic fibers, other than those occurring in the walls of blood vessels, are not evident in the placental substance. They certainly are not found in the "spirochetal" foci, and the spirochete-like objects have not been found in the walls of the blood vessels, *i.e.*, in the places where the elastic fibers are known to occur. Moreover, elastic fibers are not argentophilic.

The above observations were fully supported by study of the control syphilitic tissue.

With the above possible sources of confusion ruled out, we conclude that in so far as the purely morphological evidence can be interpreted, the observed argentophilic spiralled structures are spirochetes. They agree in shape, size and spacing of coils with the morphological characteristics of the genus *Treponema*,[19] closely resembling two of its species, *T. pallidum* and *T. calligyrum*, which are known to occur in the genital tract of man. It is not possible from the present study to identify the spirochetes observed, but the striking fact, as mentioned above, is that they are found in cases that present clinically a suspicious history, whether it be abortion, prematurity, stillbirth or fetal anomaly. If this fact be more than a mere coincidence, the spirochetes found would belong either to a species of acknowledged pathogenicity, or to a species that has been considered non-pathogenic but which might be responsible for the lesions described in this paper.

Summary and Conclusion

1. Placentas selected by the clinician because of a suspicion of syphilis have been studied by means of sections stained with hematoxylin and eosin, Weigert's elastica stain, silver impregnation, silver impregnation counterstained with Mallory's aniline blue, and silver impregnation partially desilverized by means of potassium iodide. The methods used were selected in order to facilitate the establishment of the nature of certain spirochete-like structures previously observed. The methods used are described.

2. The characteristics of the foci in which the spirochete-like objects are found have been described.

3. The possibility of the spirochete-like objects being elastic or collagenous fibers, reticular fibrils, fibrin, fibrinoid material, or cell boundaries has been ruled out.

4. It is concluded, on the basis of the purely morphological evidence offered, that the observed structures are spirochetes.

Note: I am indebted to Dr. H. G. Dorman for obtaining the placentas and for general suggestions concerning the problem; to Dr. E. Mayer for suggestions and remarks on the silver technics; to Dr. E. W. Dennis for help on the morphology of the spirochetes and the arrangement of the article; and to Mr. H. Berberian, for assistance with the microphotographs.

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DESCRIPTION OF PLATE

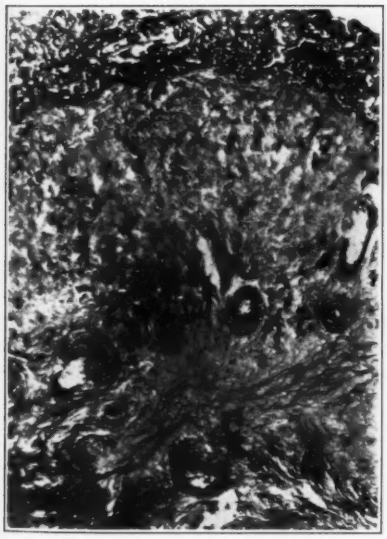
PLATE 76

FIG. 1. Microphotograph of a section impregnated with silver and counterstained with Mallory's aniline blue stain for collagenous fibers, showing (*a*) an artery with periarteritis and endarteritis with collagenous fibers partially occluding the lumen, and (*b*) the border which stains orange-red and shows spirochetes at a higher magnification.

FIG. 2. A microphotograph from the same slide as Fig. 1 showing periarteritis and endarteritis in five arterioles in a neighboring field.

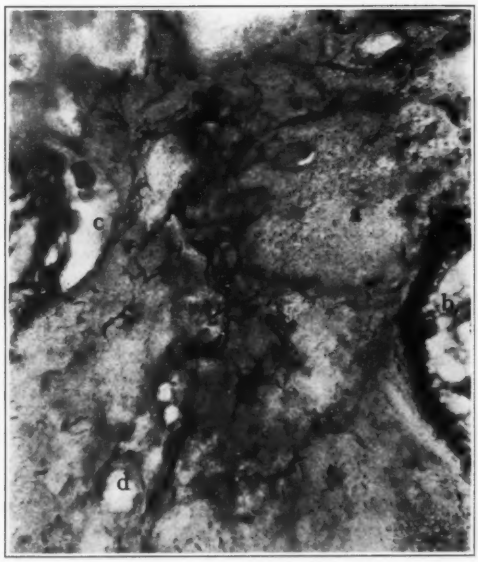
FIG. 3. A microphotograph of a preparation stained by the same technic as Figs. 1 and 2, and showing (*a*) a focus surrounded with argentophilic granules and spirochetes, (*b*) a portion of a degenerating villus, (*c*) a partially occluded blood vessel, and (*d*) small islets of collagenous fibers.

1



2

Sahyoun



3

Spirochete-Like Structures in Placenta

EXPERIMENTAL PNEUMONIA PRODUCED BY TYPHUS RICKETTSIAE *

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After Prowazek and da Rocha-Lima demonstrated that the organism of typhus fever multiplied within the cells of the intestinal tract of infected lice, it was assumed that Rickettsiae were obligate parasites of certain animal cells. This was confirmed by Wolbach and coworkers [1] who observed intracellular Rickettsiae in the endothelium of the blood vessels of humans and animals infected with typhus. A few years later Mooser [2] discovered intracellular organisms in the mesothelial cells of the tunica vaginalis of typhus infected guinea pigs, and Zinsser and Castaneda [3] cultivated Rickettsiae in large numbers in the peritoneum of rats where the organisms multiplied readily within the serosal cells. Okamoto [4] reported that he had observed Rickettsiae in the alveolar cells of the lungs of mice infected by the intraperitoneal route. Recently Hitz,[5] in our laboratory, succeeded in cultivating Rickettsiae in minced guinea pig lung suspended in an asciticserum mixture. The cultures did not grow as readily as those made from the tunica vaginalis, but his findings corroborate indirectly those of Okamoto. He observed also, in cultures made from the tunica vaginalis, typical Mooser cells in close proximity to muscle fibers suggesting a relationship with the connective tissue sheaths, although the true nature of these cells has not been determined.

In a recent preliminary report [6] we showed that mice and rats could be infected by the intranasal route and that a considerable growth of Rickettsiae could be obtained in the lung. It was also stated that the lining of the bronchi was found to be parasitized with intracellular bodies. The cells were infected in such a manner that the epithelium resembled the gastro-intestinal tract of typhus infected lice.

The various types of cells in which Rickettsiae have been found

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show that the cellular requirements of the agent of typhus are not as limited as we had assumed. In this report we wish to confirm our first experiments and give further information concerning these observations.

Material and Methods

The strain of typhus used for the present experiments was our "L" orchitic strain isolated from a case of typhus in the General Hospital of Mexico City in 1936. This strain has been transferred over 150 times in male guinea pigs which have constantly shown the typical scrotal reaction.

Adult mice and young white rats were inoculated by the intranasal route by means of a 1 cc. pipette applied to the nostrils, administering very slowly a total of 0.2 cc. to the mice and 0.4 cc. or a little more to the rats. For inoculation the animals were anesthetized with ether and the anesthesia was repeated as necessary until the whole dose of inoculum was given.

The inoculum prepared from the guinea pig material was obtained by washing the tunica vaginalis with 1 cc. of an isotonic sodium citrate solution for each guinea pig used. The washings were centrifuged at low speed in order to remove gross particles. The inoculum prepared from the infected lungs of mice or rats was obtained by grinding the lungs with powdered sterile glass and emulsifying them in saline. The lungs of mice were suspended in 7 cc. and those of rats in 30 cc. each of saline. The emulsions were then centrifuged at low speed to remove the particles of glass.

Rabbits of various sizes were anesthetized with Dial (Ciba) by the intraperitoneal route in amounts of 0.55 cc. per kilo of body weight, and others with ether. When completely anesthetized 8 to 10 cc. of the inoculum was injected directly into the trachea. When desired, the body temperature of the inoculated rabbits was kept below 37° C. by repeating the injection of Dial. Three or 4 doses, each containing 75 per cent of the original dose, applied at convenient intervals of time, were sufficient to keep the animals 72 hours or more under narcosis and at low body temperature. As stated elsewhere,[7] low body temperature favors the growth of typhus Rickettsiae.

The lungs of the animals found dead or killed at various intervals of time after inoculation were placed in sterile Petri dishes

and plain or blood agar slants were smeared with small pieces of tissue. Smears and impressions on slides were made for direct examination and fragments of the lung were fixed for histological study.

The smears were stained by Giemsa's method and by our methylene blue-safranin stain. Tissues were fixed in Regaud's solution (potassium bichromate 3 per cent, sodium sulphate 1 per cent, and formalin 10 per cent). Sections were stained with a modification of one of Pappenheim's methods, recommended by Hitz,[5] as follows:

SOLUTION A

Distilled water	100 cc.
Glacial acetic acid	1 drop
May-Grünwald stain	20 cc.

SOLUTION B

Distilled water	100 cc.
Glacial acetic acid	1 drop
Giemsa's stain	5 cc.

After treating the sections with Solution A for 15 minutes, transfer without washing into Solution B and leave for 30 minutes to 1 hour. Both solutions have a better action at 37° C. Dehydrate rapidly with absolute alcohol and after clearing in xylol mount in cedar oil. This seems to be the simplest way of staining Rickettsiae in sections.

Experimental

The inoculation into mice of washings from the tunica vaginalis of typhus infected guinea pigs by the intranasal route gives rise to fatal results in a large percentage of the animals. The mice die usually about 96 hours after inoculation and show pneumonic lesions characterized by considerable hyperemia and hemorrhage of the lungs, which usually become completely involved. The affected lobes of the lungs resemble liver or spleen. The non-affected tissue shows a compensatory emphysema. If the lungs are left in a Petri dish for a few minutes there is an exudation of blood which soon coagulates.

Microscopic examination of the lungs shows that the capillaries are filled with blood and many extravasated red cells have invaded

the alveoli. There is also an infiltration by polymorphonuclear leukocytes, which are present in large numbers but do not suggest pus formation. Many leukocytes show various degrees of necrobiosis, mainly pyknosis. The cellular degeneration seems to involve the cells of the alveoli, bronchi and capillaries. The cytoplasm of many cells is swollen by considerable numbers of small organisms. Many of these cells appear to belong to the blood capillaries, but the epithelium of the bronchi is also found to be infected with the same parasite, presenting an appearance similar to that of the intestinal tract of typhus infected lice. The infected bronchial cells, as well as those scattered in the lungs, resemble the so-called Mooser cells, so characteristic of the lesions of the tunica vaginalis and the peritoneal infection of X-rayed, typhus infected rats. Extracellular organisms may also be seen but are not very numerous. In some animals ordinary bacteria are also present. Smears or impressions made from the lungs of infected animals show large numbers of small intracellular and extracellular organisms. Many polymorphonuclear leukocytes have phagocytozed these organisms. The general appearance resembles the smears made from the peritoneal exudate of X-rayed, typhus infected rats.

The inoculation of washings from the tunica vaginalis of infected guinea pigs into rats of various sizes has given very irregular results. Only a few animals have developed lesions in the lungs after inoculation. The microscopic appearance is similar to that observed in mice and Mooser cells are easily found.

Transfer of the Lung Infection by Means of a Lung Emulsion

Rats, mice and rabbits inoculated by the intranasal route with emulsions of the lung from infected animals died within 72 to 96 hours after inoculation with extensive lesions in the lung identical with those we have described.

It has been possible to transfer the infection from rat to rat for several generations with the same characteristic hemorrhagic lesions developing in the lungs. In one instance a "lung strain" was obtained from a rat which developed lesions after infection with washings from the tunica vaginalis of infected guinea pigs. This strain which killed the animals within 4 to 6 days was unfortunately lost on the 7th transfer.

Cultures on Plain or Blood Agar from Infected Lungs

Many attempts to cultivate ordinary bacteria were made, using as a medium plain or blood agar slants. For this purpose small pieces of lung were cut as far as possible from the large air ducts and were smeared on the slants. The mice infected by washings from the tunica vaginalis of infected guinea pigs showed few or no colonies on the slants after 48 hours incubation. In rats inoculated with emulsions from the lungs of mice, contaminating organisms were rarely found, but in transfers from rat to rat ordinary bacteria, usually Gram-negative, were cultivated on various occasions. These organisms were tested with typhus serum and did not give the agglutination reaction. Emulsions of the Gram-negative bacilli were inoculated into guinea pigs intraperitoneally and produced a peritoneal infection with death of the animal in from 48 hours to 8 days. The exudate showed the injected organisms, but Mooser cells were not found. The inoculation of large doses of the same Gram-negative bacteria into rats by the intranasal route failed to produce the hemorrhagic pneumonia shown by those inoculated with typhus material or with emulsion from lung transfers.

Resistance of Rats and Mice to a Second Intranasal Inoculation

The rats and mice which survived inoculation with either washings from the tunica vaginalis or emulsion of the lungs from infected mice were reinoculated 10 to 15 days later with an emulsion of the lungs from an infected mouse by the intranasal route. These animals survived the test.

Identification of the Etiological Agent Producing Hemorrhagic Pneumonia in Mice, Rats and Rabbits Inoculated with Typhus Material

Several guinea pigs were inoculated intraperitoneally with emulsions made from lungs showing typical hemorrhagic lesions and many Mooser cells. The material was obtained from rats infected with virus from the 3rd to the 7th transfer from lung to lung. As a control, typhus immune guinea pigs were injected with the same material.

In one instance both normal and immune guinea pigs died on the 3rd or 4th day after inoculation with a peritoneal infection by a Gram-negative bacillus similar to that isolated on agar cultures. No bodies resembling Rickettsiae were observed.

Two normal guinea pigs showed fever and swelling on the 4th day after inoculation and one died from peritonitis on the 6th day. The other animal was killed and material was transferred into another guinea pig. Smears from the tunica vaginalis showed typical Mooser cells, but a few bacteria were also seen. The guinea pigs inoculated with this material developed a peritoneal infection with a Gram-negative organism. The typhus immune guinea pig controls also died with peritonitis.

The inoculation of emulsion of the lung of the 5th generation from rat to rat produced in a normal guinea pig typical fever and swelling without intercurrent infection, while the typhus immune controls showed no fever or swelling. These animals were tested later with the "L" strain and showed no reaction at all.

A normal and an immune guinea pig were inoculated with an emulsion of the brain from a rat which was previously inoculated with washings from the tunica vaginalis of guinea pigs inoculated with material from the lung. The normal animal developed typical fever and swelling and was found to be immune when reinoculated with the "L" typhus strain. The immune guinea pig showed no reaction at all.

Guinea pigs were vaccinated with 2 doses of 1 cc. each, given subcutaneously, of an emulsion of organisms obtained from the lungs of infected rats. The suspensions made in formalinized saline were purified by fractional centrifugation and contained 3×10^9 organisms per cc. When the guinea pigs were tested 15 days after the first vaccination they were found to be immune to the "L" strain.

Purified emulsions of organisms found in the lungs of infected rats were submitted to microscopic agglutination tests. These were made by mixing a droplet of serum with a drop of the emulsion and adding a little methylene blue. The mixtures were placed in a hanging drop preparation and observed under the No. 40 objective. The mixtures containing normal human or guinea pig serum showed no agglutination. Those containing human convalescent typhus serum or immune guinea pig serum were aggluti-

nated within a short time. Care was taken to use the serums of guinea pigs bled before and at various intervals of time after the inoculation with orchitic typhus.

To this data we may add the further information that guinea pigs inoculated with minute amounts of emulsion of the lungs from mice inoculated with washings from the tunica vaginalis of guinea pigs, and from rats infected from such mice, invariably develop typical typhus infection.

From these various experiments we conclude that the organisms found in the lungs of rats and mice infected with typhus material and transmitted by emulsions of the lung to other animals are *Rickettsiae prowazeki* which grow in great numbers in the cells of the bronchi, the alveoli and the endothelium of the capillaries. The contaminating organisms are easily detected by cultivation on agar slants and inoculation into animals. These contaminants have a tendency to increase in proportion with the transfers from rat to rat and may finally predominate in the lungs, but are rare in mice infected with material from guinea pigs and in rats inoculated with emulsions from the lungs of mice.

Production of Large Quantities of Rickettsia Bodies from Infected Lungs

When a mouse is inoculated with washings from the tunica vaginalis of typhus infected guinea pigs sufficient amounts of Rickettsiae are produced to infect 15 medium sized rats. Whenever the infection is successful the mice die 96 hours after inoculation and the rats infected with emulsion from the lungs die with great regularity on the 3rd day after inoculation.

Rabbits were anesthetized with Dial and inoculated with an emulsion of the lungs from infected rats. Each rabbit received about one-third of a whole lung. The animals were kept at low body temperatures, which is essential to obtain abundant growth of Rickettsiae.[7] Rabbits inoculated by the intratracheal route but not submitted to a depression of temperature, developed a fatal disease with considerable involvement of the lungs and showed Rickettsiae in large numbers, but the yield was negligible compared to that obtained from animals subjected to a low body temperature.

From the lungs of medium sized rats we obtained, after grind-

ing and purifying by fractional centrifugation to remove foreign particles, about 10 gm. of packed Rickettsiae per 100 animals. This may be diluted to 5 liters or more to obtain a concentration of Rickettsia bodies suitable for vaccination. Two technicians may easily inoculate 3 lots of 100 rats a week to obtain about 15 liters of vaccine.

We have not so far calculated the production that may be obtained from rabbits kept at low body temperatures by continued narcosis with Dial, but roughly one may estimate that as much vaccine can be obtained from 1 rabbit as may be obtained from 10 rats.

The various methods of production of the Mexican vaccine first prepared by Zinsser and Castaneda enable us to obtain sufficient amounts of formalin-killed Rickettsiae which may be used advantageously as a prophylactic means against typhus.

Summary

The intranasal inoculation of mice and rats with typhus virus (orchitic variety) has given rise to hemorrhagic lesions of the lungs which kill mice in 96 hours and rats in 72 hours each. The lungs show in sections and smears considerable numbers of Rickettsia bodies which have been obtained in pure suspension by grinding and fractional centrifugation. Rabbits have also been infected by the intratracheal route with or without forcing down the body temperature. The animals develop hemorrhagic pneumonia, and Rickettsiae are present in large numbers in smears and in sections of the lungs, but the animals subjected to a low body temperature produce greater quantities of Rickettsia bodies. These rabbits die in from 48 to 96 hours after inoculation. The rabbits not submitted to a low body temperature die after a longer period of time and show lesions of the lungs which are more extensive but which contain fewer Rickettsiae.

To produce massive infection of the lungs it is necessary to inoculate considerable numbers of Rickettsiae.

This method of cultivating Rickettsiae has proved very useful for obtaining typhus vaccine for practical purposes.

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DESCRIPTION OF PLATE

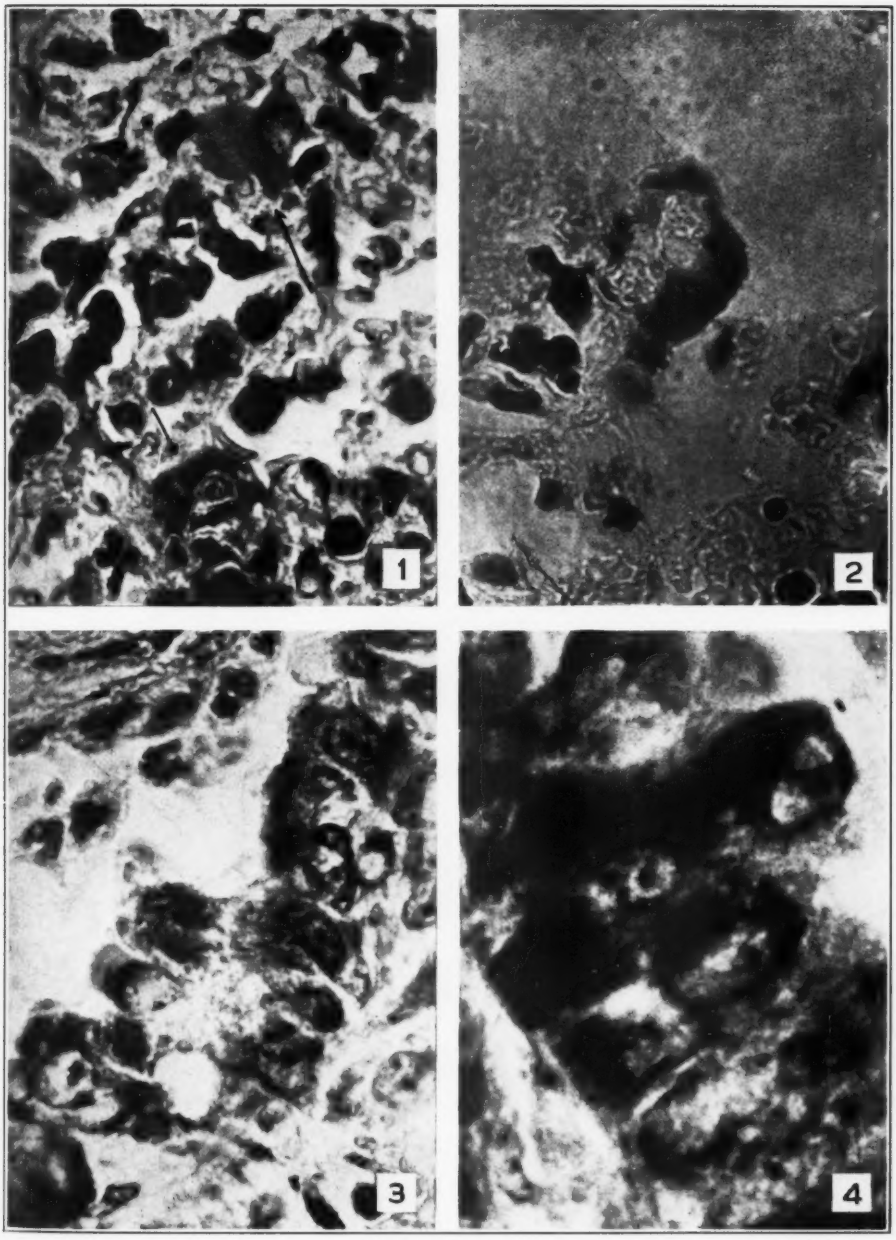
PLATE 77

FIG. 1. Lung of rat infected with typhus Rickettsiae showing two Mooser cells indicated by the arrows.

FIG. 2. Parasitized cells from the lung of a rat, apparently from a capillary.

FIG. 3. Section showing the bronchial epithelium parasitized with Rickettsiae.

FIG. 4. Higher power of Fig. 3 showing bronchial cells filled with Rickettsiae.



Castaneda

Pneumonia Produced by Typhus Rickettsiae

HISTOPLASMOSIS IN INFANCY *

Report of a Case

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Histoplasmosis is a rare mycotic infectious disease. Six cases are usually cited in recent texts. A review of the recent literature indicates that 3 additional cases can be added — 1 by Hansmann and Schenken,[1] 1 by Phelps and Mallory,[2] and 1 by Müller.[3] The following case which came to our attention makes the 10th case of human histoplasmosis reported to date.

Report of Case

Clinical History: A female infant was delivered by cesarean section in Detroit. Shortly after birth the infant was removed to Missouri for a time. After returning to Detroit she developed a chronic respiratory condition characterized by intermittent paroxysms of coughing which was diagnosed as pertussis. The child developed a left otitis media followed by a persistent serosanguineous discharge. The respiratory symptoms persisted for 9 weeks, at which time the mother became alarmed because of the child's pallor and the enlarging abdomen. The infant was hospitalized and the anemia was interpreted as secondary to some infection. A roentgenogram of the thorax showed indefinite peribronchial infiltration near the hilum of each lung.

The infant was removed to another institution where she was hospitalized for 57 days. Here she ran a continuous febrile course and the paroxysms of coughing continued. The abdominal enlargement increased and was found to be due to a hepatosplenomegaly.

Blood studies showed a constant low erythrocyte count averaging 2,000,000 per cmm., and hemoglobin values which averaged slightly less than 50 per cent. There was a constant leukopenia, the leukocyte count averaging 1500 cells per cmm. The differential counts showed the neutrophilic leukocytes to average between 45 and 55 per cent, lymphocytes 40 and 60 per cent, and monocytes 2 and 12 per cent. Numerous normoblasts were present. This blood picture persisted in spite of repeated small transfusions of blood and therapy with liver extract and pentnucleotide.

On the basis of a clinical diagnosis of splenic anemia, splenectomy was performed. Because of the poor physical state of the patient no additional exploration was attempted.

Subsequent to the splenectomy there was definite improvement in the blood picture: the erythrocyte count rose steadily to 3,500,000, the hemoglobin rose to 60 per cent, and the leukocyte count rose to 5500. However, the fever, weakness and abdominal enlargement persisted.

* Received for publication April 4, 1939.

The child died at the age of 8 months, 3 weeks after the splenectomy and about 4 months after the onset of symptoms. At no time was jaundice noted. Slight edema of the lower extremities was present at intervals. During the last 10 days of life some stiffness of the neck without rigidity was present, but the Kernig test was negative. The spinal fluid was under slightly increased pressure and contained 1 lymphocyte per cmm.

Comment

The exact nature of the disease was not recognized until several months after the death of the child when the pathological condition present was identified by a histological examination of the spleen. Permission for an autopsy was refused.

The spleen removed at operation weighed 159 gm. The organic form was preserved and the capsule was thin. On section the pulp was rather firm in consistence and a grayish pink in color. The malpighian corpuscles were not prominent.

The most striking pathological feature seen on microscopic examination was the tremendous proliferation of reticuloendothelial cells (Fig. 1). The pulp was crowded with great numbers of large cells of the macrophage type, many of which contained clusters of round or slightly oval bodies (Figs. 2 and 3). These bodies were made up of a thick, clear, non-chromatic capsule surrounding a finely granular cytoplasm. The chromatin was ordinarily aggregated at one pole of the cell and was often arranged as a crescent or occasionally as a compact dot located near the capsule of the cell. A blepharoblast was not demonstrable in these parasites, a feature differentiating this organism from the Leishman-Donovan body. The number of parasites within individual macrophages varied from a few to as many as 25. Frequently they occurred in clusters, suggesting a mulberry arrangement with no remnant of the macrophage to be seen. The splenic blood sinusoids contained a large number of macrophages, some of which had phagocytozed variable numbers of parasites. Most of the red blood corpuscles in the splenic pulp were hemolyzed, and phagocytosis of blood pigment was prominent. Some macrophages contained both parasites and blood pigment.

Subsequent to the discovery of the parasites in sections of the spleen two blood films which had been prepared during the last week of illness were reexamined. In these blood smears the parasites were found phagocytozed in large mononuclear cells (Fig. 4) and occasionally in neutrophilic polymorphonuclear leukocytes.

Discussion

All of the reported cases of histoplasmosis have terminated fatally; in only 2 cases was the nature of the disease recognized before death so that cultural studies could be made. We are indebted to DeMonbreun [4] for his classical study of the infective agent isolated from the case reported by Dodd and Tompkins.[5]

As yet, mycologists do not agree as to the exact classification of this yeast-like fungus. If we accept the observations of Moore,[6] which are supported by Dodge,[7] the fungus recovered from the case reported by Dodd and Tompkins and that from Hansmann and Schenken's case belongs to the genus *Histoplasma* (*Posadasia*) of the family *Coccidioideaceae*. Two species of the genus Histoplasma are recognized, *Histoplasma capsulatum* and *Histoplasma pyriforme*. DeMonbreun and the Italian workers, Redaelli and Cifarri,[8] do not believe that the Histoplasma fungus produces asci and the latter two workers prefer to group Histoplasma with Cryptococcus.

The epidemiology and pathogenesis of histoplasmosis await further study. The occurrence of respiratory disturbances in 6 of the reported cases suggests that the portal of entry into the human host is most likely through the respiratory system. This finds additional support from the early clinical picture in our case. The infant exhibited early and persistent respiratory symptoms in the nature of intermittent paroxysms of coughing which simulated pertussis. In the case reported by Dodd and Tompkins similar respiratory symptoms were present. On the other hand, the prominent intestinal disturbances in Müller's case and the dominance of granulomatous lesions in the intestine and mesenteric lymph nodes in the case reported by Crumrine and Kessel,[9] suggest that the portal of entry may at times be through the intestinal mucosa. The association of intestinal ulcers in 2 of Darling's cases,[10] and also in Müller's case, tends to support this view. Again, it is possible that the portal of entry may rarely be through the skin. In the case reported by Hansmann and Schenken there was a protracted, chronic papulopustular dermatitis from which Histoplasmata were recovered.

Irregular fever, weakness, anemia, leukopenia and splenomegaly are the features usually described in the clinical course of the disease.

Pathologically the most prominent feature is the marked reticuloendothelial proliferation, especially in the spleen, lymph nodes, liver and bone marrow. Large numbers of macrophages are formed which engulf the parasites. There is a tendency for pseudotubercles to be produced in the lungs, and in 3 of the reported cases superficial ulcers of the intestine were observed.

Case reports of histoplasmosis indicate that the parasite may be found in the epithelial cells of the intestine and the bronchi, and even in the cortical cells of the adrenal gland. In the adrenal, caseous lesions simulating tuberculosis may be produced (Hansmann and Schenken [1]).

The accumulated data, however, indicate that the classical features of the disease are subject to considerable variation. Thus anemia was not a feature of Hansmann and Schenken's case, and leukocytosis was present in the cases reported by Phelps and Mallory, Hansmann and Schenken, and particularly in that reported by Dodd and Tompkins. Splenomegaly was absent in 2 cases, those of Hansmann and Schenken, and Crumrine and Kessel. The one feature common to all was the presence of the characteristic clusters of phagocytozed yeast-like fungi in reticuloendothelial cells of various organs.

Redaelli [11] worked with laboratory animals which had been inoculated with cultures of the organisms recovered from the case reported by Hansmann and Schenken. His experimental studies demonstrate an extreme degree of proliferation of reticuloendothelial cells in the lymph nodes, spleen and bone marrow, and an active phagocytosis of the organism by macrophages. In the early course of the experimental cases phagocytic activity was pronounced. However, sooner or later there appeared to be a collapse of this phagocytic power, and in those animals that survived infection with the saprophytic form of the fungus there remained continued impairment of phagocytic function as determined by the Congo red test.

Summary

A fatal case of infantile histoplasmosis is reported. Apparently this is the 10th case of human histoplasmosis to be recorded, and the 2nd case observed in an infant.

The clinical manifestations were chronic paroxysmal cough,

anemia, leukopenia, continuous fever, weakness and hepatosplenomegaly.

Of the organs, only the surgically removed spleen was available for study. This showed a marked proliferation of reticuloendothelial cells, many of which contained the fungus.

Phagocytic cells laden with parasites were found in blood smears from the circulating blood.

The Histoplasma is a fungus, the pathogenic form of which resembles yeast. The saprophytic form is a myceliate spore-bearing fungus whose exact taxonomic position is still in question.

Knowledge regarding the epidemiology and pathogenesis of histoplasmosis is incomplete.

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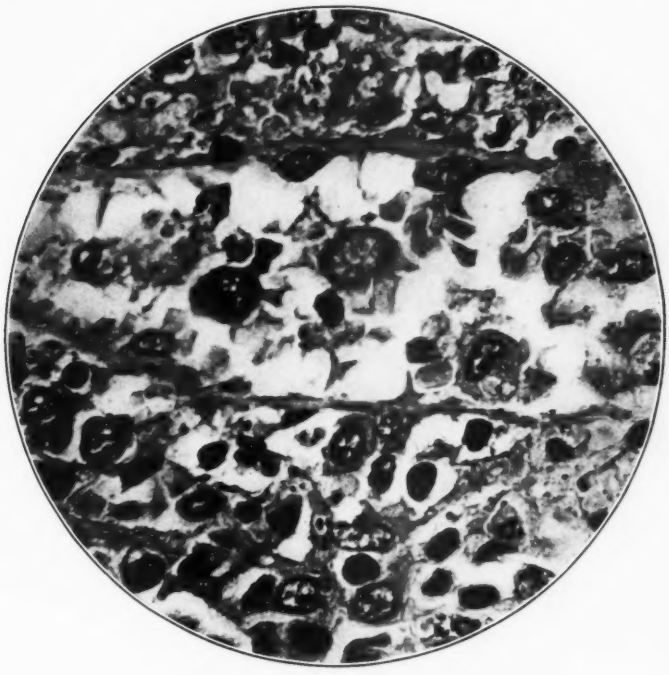
DESCRIPTION OF PLATES

PLATE 78

FIG. 1. A splenic blood sinusoid with surrounding red pulp showing reticuloendotheliosis. Numerous encapsulated blastospores in small and large clusters are seen. Macrophages are present in the sinusoid, many of which show degenerative changes. Iron hematoxylin and Masson's trichrome lichtgrün stain. × 1000.

FIG. 2. A splenic blood sinusoid containing macrophages. Clusters of *Histoplasmata capsulata* are seen in the degenerated pulp cells. Iron hematoxylin and Masson's trichrome lichtgrün stain. × 1400.



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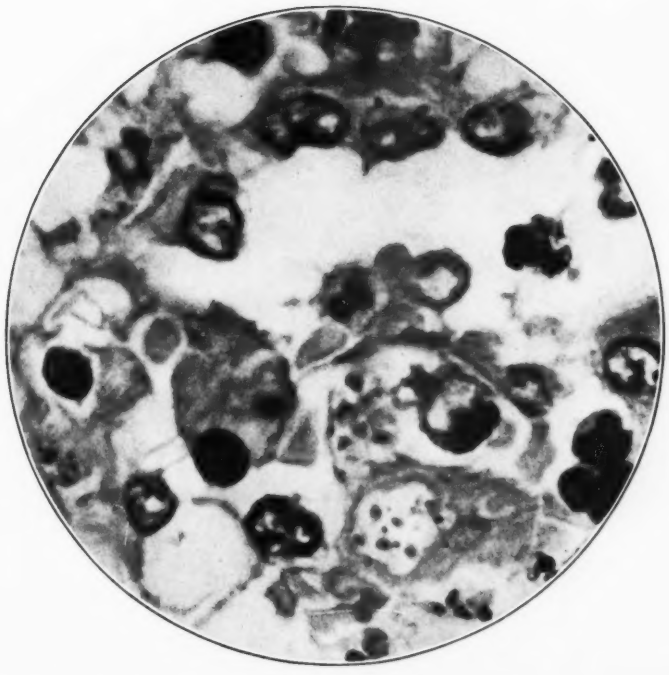

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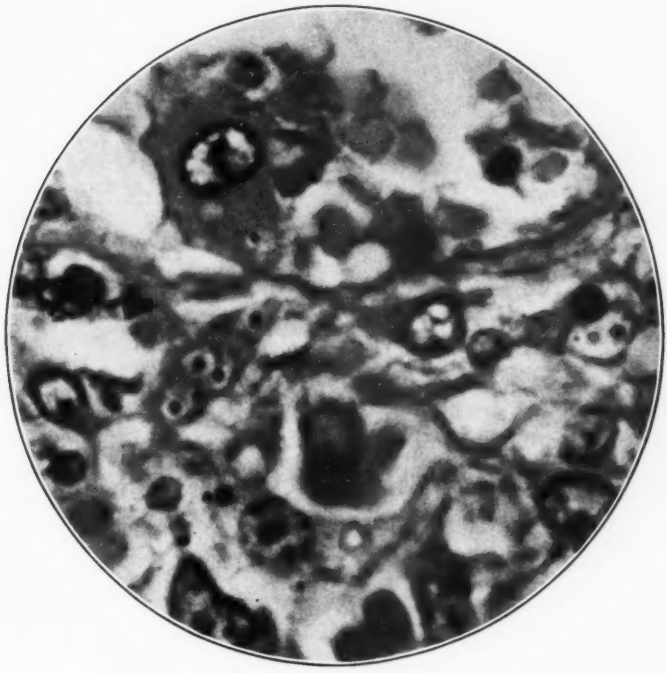
PLATE 79

FIG. 3. A degenerating macrophage in a splenic blood sinusoid is shown on the right. A cluster of *Histoplasmata capsulata* is present in a degenerated macrophage in the pulp. Iron hematoxylin and Masson's trichrome lichtgrün stain. × 1400.

FIG. 4. A macrophage in the peripheral blood showing a large cluster of *Histoplasmata capsulata* in the cytoplasm crowding and deforming the cell nucleus. The typical mulberry-like formation is seen. Wright's blood stain. × 1400.



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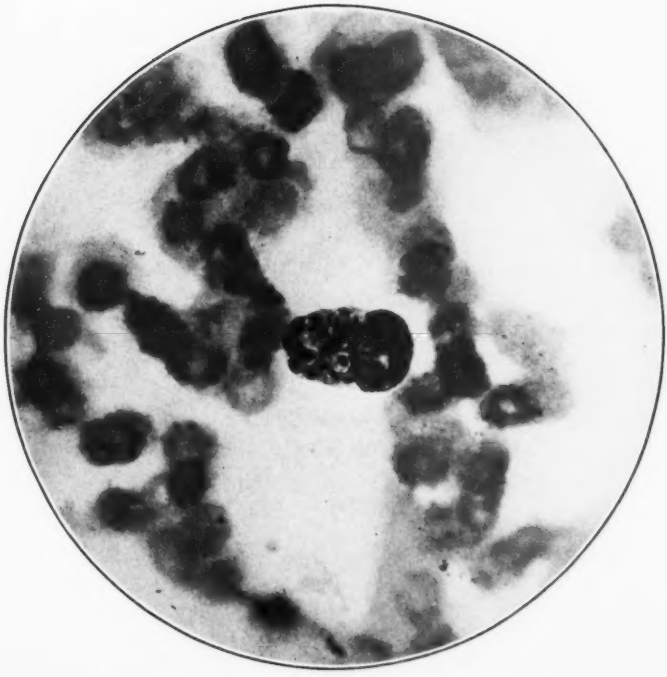

4

MALABSORPTION OF FAT (INTESTINAL LIPODYSTROPHY OF WHIPPLE)*

Report of a Case

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In 1907 Whipple [1] reported a fatal case of "a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues." The patient was a physician, aged 36 years. The clinical course of the disease was characterized by recurring attacks of arthritis, progressive emaciation, enlargement and tenderness of the abdomen and fatty diarrhea. At autopsy neutral fat and fatty acid deposits were found in the intestinal mucosa and in the mesenteric and retroperitoneal lymph nodes. The mesenteric lymph nodes were greatly enlarged, some measuring 3 to 4 cm. in diameter. The cut surfaces of the nodes presented an opaque pale yellow color with almost complete disappearance of the glandular tissue. The retroperitoneal lymph nodes were of similar character. The microscopic appearance of the jejunum, ileum and the glandular lesions may be summarized as follows: The villi were enlarged. The lymphatic channels were dilated and filled with large fatty masses. There were large numbers of "polyblasts and large mononuclear ameboid cells with a pink granular protoplasm. Large 'foam' cells were present. Examination of the lymph glands revealed the process to begin in the sinuses with invasion of the characteristic cells and small irregular fat deposits. The final stage presented a very large gland packed with fat deposits of all sizes and shapes, whose stroma was made up of dense fibrous tissue full of ecchymoses and great numbers of giant and mononuclear cells." Whipple suggested that the term "intestinal lipodystrophy" be given the newly described disease.

Blumgart [2] in 1923 reported 3 fatal cases of malabsorption of fat in adults. All of the cases presented indefinite clinical outlines. The occurrence of fatty stools, great loss of weight and strength,

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and enlarged mesenteric lymph nodes containing fatty substances, suggested a relation to the case described by Whipple. None of Blumgart's patients had arthritis, eosinophilia, purpura or enlarged intestinal villi, but all showed the significant anatomical deposits of fat confined to the intestinal mucosa and the mesenteric lymph nodes. Small granular elevations, gray in color, were present in the mucosa of the small intestines. On microscopic examination these lesions were found to consist of groups of phagocytes containing ingested fat. The phagocytes were large and mononuclear, and contained a foamy reticulated cytoplasm. The mesenteric lymph nodes were noticeably enlarged and hyperplastic and contained similar phagocytes.

Jarcho [3] in 1936 reported a case of steatorrhea with unusual intestinal lesions, which he described as being clinically similar to and anatomically identical with the case reported by Whipple. Jarcho reviewed the cases of Whipple and Blumgart. He classified Blumgart's 2nd case as analogous to the case reported by Whipple. Jarcho stated that these 3 cases were characterized by "unusual and dense deposits of fat throughout the length of the jejunum and ileum and in the mesenteric lymph nodes with concomitant infiltrates of mononuclear cells and giant cells; the latter were frequently found applied to the margins of fatty deposits. There were no morphological changes in the pancreas and there was no evidence of active infection, tuberculous or other."

Boeck, in discussing a paper by Bargen, Bollman and Kepler [4] on the diarrhea accompanying pancreatic insufficiency, described a case quite suggestive of intestinal lipodystrophy. The patient was a 45 year old physician who had persistent steatorrhea, abdominal distention and a moderate secondary anemia. A laparotomy was done and all the mesenteric glands were found enlarged. Microscopic examination of one of the lymph nodes revealed almost complete replacement of lymphoid tissue with "reticulocyte cells" filled with fat, and deposits of cholesterol crystals. At autopsy fat and cholesterol deposits were also found in the walls of the small intestine, with some mucosal atrophy.

Through the courtesy of Dr. W. B. VandeGrift of the Department of Pathology of The Johns Hopkins University, tissue slides from the following case were compared with those from the cases

of Whipple and Jarcho and the opinion was expressed that the anatomical lesions were of the same basic type.

Report of Case

Clinical History: A 74 year old retired carpenter was admitted to the Starling-Loving University Hospital with the complaint of an enlarged abdomen and constipation. He had been in good health until about 1 year previous to admission, at which time he became conscious of abdominal discomfort after meals. Because of postprandial discomfort he decreased his food and liquid intake. He lost 35 to 40 pounds in weight during the preceding year. Pitting edema of the extremities, dyspnea on slight exertion and orthopnea developed during the 3 weeks previous to admission. He had had a moderately severe generalized pruritis, most marked in the evening. There was no history of jaundice or of diarrhea. Over a period of 30 days in the hospital 36 stools were passed which varied in frequency from 5 in 1 day to none in 2 days. None were passed during 11 of the 30 days. The stools varied from a large to a moderate amount, were liquid to soft in consistence, and were of a light brown to brown color. Apparently they were not sufficiently unusual to attract clinical attention.

Physical Examination: The patient was an emaciated elderly male, moderately orthopneic and dyspneic. The temperature was normal. Superficial lymph nodes were palpable and the cervical lymph nodes were slightly enlarged. The mouth was edentulous and the tongue smooth but not atrophic. Crepitant râles were heard throughout the bases of both lungs. The pulse frequency was 80, the rhythm regular and the volume of good quality. A blowing systolic murmur was localized at the mitral area. The abdomen was distended, dull to percussion and a definite fluid wave was elicited. A mass was palpable in the left upper quadrant, which was firm in consistence, irregular in outline and extended 3 cm. below the costal margin. The liver was palpated 3 cm. below the right costal margin. There was a reducible left indirect inguinal hernia containing a portion of intestine. The lower extremities were edematous and pitted on pressure. The skin was rough and dry and there was no abnormal pigmentation present. The hair was dry and lusterless and the nails and lips slightly cyanotic. The eyes reacted to light and accommodation. There was a moderate tortuosity of retinal vessels. The neurological examination revealed no noteworthy abnormalities. Rectal examination revealed a few hemorrhoidal tags and a spastic sphincter. The prostate gland was firm but not definitely enlarged.

Clinical Laboratory Investigations: The erythrocyte count on admission was 3,400,000 per cmm., and the total leukocyte count 10,700 per cmm. A differential count revealed 25 per cent neutrophiles, 59 per cent lymphocytes and 16 per cent monocytes. The blood platelets were 496,400 per cmm. The erythrocytes comprised 39 per cent and the leukocytes 1 per cent of the packed cell volume. The actual sedimentation rate (Wintrobe) was 0.4 mm. per minute; the corrected sedimentation rate was 0.2 mm. per minute. Red cell fragility (Wiseman technic) ranged from 0.43 to 0.30. Urinalysis and renal function tests (concentration, dilution and phenolsulphonphthalein) were essentially normal. The blood urea nitrogen was 9.2 mg. per cent. A liver function test with bromsulphalein revealed a 90 per cent retention in 5 minutes

and less than 10 per cent retention in 30 minutes. The Wassermann and Kahn tests of the blood serum were positive (4 plus).

Complete hematological investigations were conducted daily. The total leukocyte count ranged from 10,700 per cmm. to 24,000 per cmm.; absolute counts ranged as follows: lymphocytes 6420 to 20,640 per cmm., monocytes 900 to 2288 per cmm., and granulocytes 1680 to 3900 per cmm. The erythrocyte count averaged 3,300,000 per cmm., and the hemoglobin varied from 10 to 12.8 gm. (Newcomer). The reticulocytes averaged 1.2 per cent. The blood platelets varied from 212,100 to 645,840 per cmm. The icterus index was 7, and the direct van den Bergh test was negative; the serum bilirubin was 0.3 mg. per cent.

Clinical Course: The patient's temperature fluctuated between 95.8° and 99° F. Five paracenteses were done for relief of abdominal distress, and approximately 2500 cc. of cloudy straw colored fluid were removed. Microscopic examination of the ascitic fluid by supravital technic revealed a preponderance of small lymphocytes, with large vacuolated clasmatocytic elements in every oil immersion field. There were 3 to 6 red blood cells per high power field, and an occasional polymorphonuclear leukocyte. In addition, an occasional large round cell with a large vesicular nucleus containing one or two prominent nucleoli, and a highly vacuolated cytoplasm containing refractile bodies was present. A biopsy of a small axillary lymph gland, measuring 1 cm. in diameter, revealed a moderate hyperplasia of lymphocytes without the characteristic appearance of lymphocytic leukemia.

After 30 days in the hospital the patient was discharged. Five days later he returned with marked distention of the abdomen, shortness of breath and urinary frequency. His temperature was normal and the pulse frequency was 100 per minute. About 4 liters of thin cream colored fluid were removed by abdominal paracentesis. Five days later about 8 liters of fluid of the same character were removed. On the 10th hospital day his pulse suddenly became weak and he became cyanotic and expired.

Abstract of Autopsy Report *

The abdominal cavity contains a large amount (6000 cc.) of cream colored fluid of milky consistence. The peritoneum is granular and the omentum is adherent to the anterior abdominal wall. There are numerous adhesions between loops of the small intestines and the colon which are easily separated. There is a diffuse plastic exudate approximately 2 mm. in diameter covering the exposed peritoneal surfaces of the intestine and the mesentery.

The liver extends 5 cm. below the costal margin in the right mammary line. The edge of the liver is rounded and the surface is diffusely and finely granular. It weighs 2360 gm. and the cut surface is of a pale brown color and presents the fine lobulation seen in portal cirrhosis.

The spleen is 3 cm. below the costal margin and there are ad-

* No. 436–999.

hesions between the omentum and the capsule of the spleen. The spleen weighs 1260 gm. and on cut section presents multiple infarctions. The capsule is slightly thickened and the pulp is moderately firm with irregular areas of hemorrhage.

Lymph Nodes: There is a marked enlargement of the lymph nodes at the root of the mesentery and those of the entire peripancreatic group. The nodes vary markedly in size from 1 to 4 cm. On cut section the glands bulge beyond their capsules. The cut surface presents numerous small cysts, from which may be expressed small fatty granules and a semisolid material. The cut surface presents a fairly uniform, creamy yellow appearance with occasional granules which are of a deeper yellow color. The larger nodes have entirely lost their normal appearance. The cut surfaces of the smaller nodes present small yellow granules embedded in the glandular tissue. The retroperitoneal group of lymph nodes (Fig. 1) extending from the coeliac axis to the brim of the pelvis are most markedly enlarged. Many of these nodes are the seat of recent hemorrhage. The mediastinal group of lymph nodes, although moderately enlarged and of similar appearance, are much smaller than the mesenteric and the retroperitoneal group. The superficial lymph nodes are grossly uninvolved by this process. The thoracic duct was not dissected out.

The mucosa of the jejunum and the ileum presents a diffuse, beefy red granular congestion without ulceration or gross deposits of material comparable to that present in the lymph nodes.

The kidneys weigh 410 gm. The surfaces are slightly granular and the cortex slightly thickened, measuring 1 cm. in diameter.

Anatomical Diagnoses: Chylous ascites, with plastic peritonitis; massive fat accumulations in mesenteric and retroperitoneal lymph nodes; portal cirrhosis, hepatomegaly; splenomegaly, with multiple infarcts; anemia; adenocarcinoma of prostate; nephrosclerosis; edema of lower extremities; left inguinal hernia; and atrophy of left testicle.

Microscopic Examination

Small Intestines: The villi are markedly broadened and blunted. The epithelium covering the villi is largely absent but well preserved when present. The outstanding characteristic of the villi is the marked dilatation of the lymphatics which with the hema-

toxylin-eosin stain presents the appearance of large empty spaces lined with endothelium. The stroma contains a large number of cells which are predominantly small lymphocytes. The serosa is covered with a fibrinous exudate in the meshes of which are numerous mononuclear cells which vary in appearance from the usual monocyte to large vacuolated macrophages of the foam cell type.

Mesenteric Lymph Nodes: The normal architecture of the lymph nodes is absent. The nodes contain numerous dilated spaces (Figs. 2 and 3) presenting the general aspects of lymph sinuses. These sinuses are more markedly dilated than in the cases of Jarcho and Whipple. They are filled with large, amorphous agglomerations, which when stained with sudan III, scharlach R and the Nile blue sulphate stain reveal the characteristic reactions for fat. Surrounding these fatty agglomerations are numerous vacuolated mononuclear macrophages and multinuclear giant cells (Fig. 2). Many of the spaces contain masses of these large foam cells without the fatty agglomerates (Fig. 3). The nuclei of the foam cells (Figs. 4–7) are frequently pyknotic, suggesting degenerative changes. The fat tissue surrounding the lymph nodes, blood vessels, pancreas and kidney contains numerous small lymphocytes presenting a leukemic type of infiltration. There are also focal aggregates of proliferating monocytic cells encountered in the kidney and in the mesenteric fat.

Pancreas: The parenchymatous cells and islands of Langerhans appear normal. There is a moderate dilatation of lymphatic vessels and pancreatic ducts, and focal areas of metaplasia of the epithelium of many of the small pancreatic ducts. There is a slight interstitial fibrosis of the pancreas and lymphocytic infiltration of the peripancreatic fat.

Liver: A moderate proliferation of bile ducts with portal fibrosis and a marked infiltration of lymphocytes is seen. There is no evidence of xanthomatous biliary cirrhosis.

Spleen: Marked passive congestion, hemorrhage, and numerous infarcts of varying ages are present. There is no evidence of nests of xanthomatous cells or deposits of fat with foam cells similar to those seen in the lymph nodes.

Special Stains: Numerous tissues were stained for *Treponema pallidum* but none was demonstrated. After comparison of sections

of lymph nodes stained for fat from this case with those from the cases of Whipple and Jarcho, Dr. VandeGrift noted that our case "contained a large amount of hyaline appearing material which takes a minimal fat stain, but is apparently lipoid substance as there are small globules of deep staining fat in this material."

Discussion

The early clinical manifestations of this case were predominately those of a blood dyscrasia, which was carefully studied in its hematological aspects. These studies led to a tentative diagnosis of a benign pseudoleukemic lymphocytosis, relative neutropenia, and a moderate hypochromic microcytic anemia. With the development of chylous ascites an obstruction of the thoracic duct due to lymphadenosis or carcinoma was postulated. Unfortunately the thoracic duct was not dissected out at autopsy. However, in the case reported by Whipple, and the 2nd case of Blumgart's series, the thoracic duct was dissected out and no anatomical obstruction was demonstrated.

In contrast with the cases reported by Whipple, Blumgart, and Jarcho, fatty diarrhea was not a prominent clinical symptom in our case and there was no evidence of rheumatic fever.

The massive deposition of fat in the sinuses of the mesenteric and retroperitoneal lymph nodes represents a quantitative increase of lipids. While obstruction of the thoracic duct may constitute the basis of such a condition, gross anatomical evidence of such obstruction is not available. Cases in which anatomical obstruction of the thoracic duct have been demonstrated have presented the clinical manifestations of steatorrhea without anatomical evidence of depositions of fat in the lymph nodes.[5]

Dilated lymphatics and lymph sinuses with massive deposits of fat, largely confined to the mesenteric and retroperitoneal lymph nodes, would scarcely be expected to originate from inadequate intestinal absorption, but malabsorption may later supervene. Although primary lymphatic obstruction cannot be eliminated, an analogous functional result may follow increased intestinal absorption of fat. This predicates increased amounts of fat in the intestines from food intake, intestinal excretion of fat, or impaired intestinal cholesterol destruction. Recent studies of lipid metabolism suggest that the "enormous increase in the amount of fat in

the feces, as in obstructive jaundice, may be due largely to increased excretion of fat rather than to diminished absorption, as was formerly believed to be the case." [6] Emaciation and depletion of body fat, one of the outstanding clinical manifestations of these cases, suggest the possibility of increased excretion of fat into the intestines. Inadequate data are available to suggest a change of the bacterial flora of the intestines which might increase cholesterol absorption. The evidence suggests massive excretion of fat into the intestines and an increased reabsorption of fat from the intestines. The term "intestinal lipodystrophy" as suggested by Whipple [1] would seem to be most appropriate.

The relation of this condition to the xanthomatous diseases seems less marked. Thannhauser and Magendantz [7] in their investigations of the xanthomatous diseases suggest that the basic disturbance lies in the xanthoma cell rather than in intermediary cholesterol metabolism. Thus, the xanthomatous nodules are not produced by a deposit of cholesterol outside the cells, but by a disease of certain cells which contain cholesterol intracellularly. In contrast with the xanthomatous diseases these cases present large aggregates of extracellular as well as intracellular fat. Furthermore, a fundamentally different type of cell is probably involved (Figs. 4–7).

Summary

A case of intestinal lipodystrophy analogous to that first described by Whipple is reported. Five similar cases have been previously reported.

Intestinal lipodystrophy is characterized by progressive emaciation, chylous ascites, fatty diarrhea, mild hypochromic microcytic anemia, a characteristic anatomical pattern and a fatal termination.

The anatomical pattern consists of the presence of large aggregations of hyaline and fatty material surrounded by large foam cells in dilated sinuses of the mesenteric and retroperitoneal lymph nodes, and the mucosa of the small intestines. These lymph nodes are markedly enlarged, and the lymphoid tissue is largely replaced as a result of this process.

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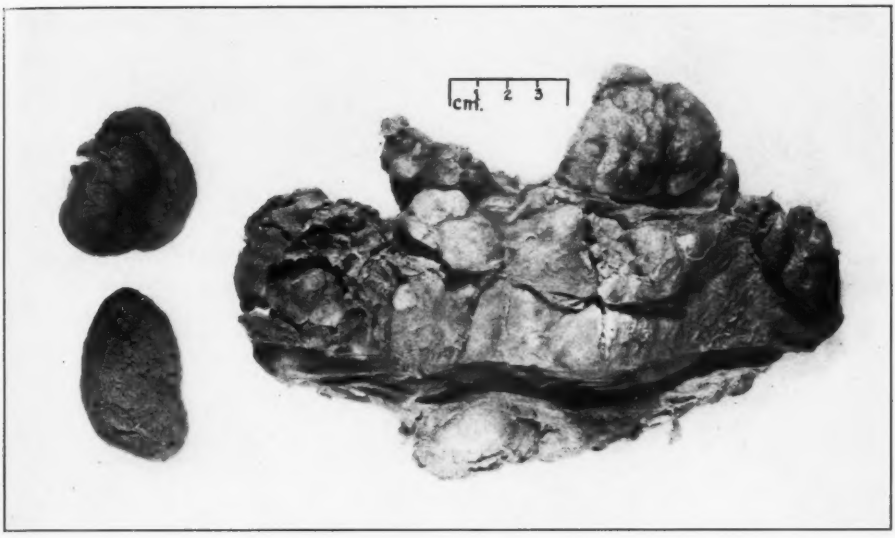
DESCRIPTION OF PLATES

PLATE 80

FIG. 1. A large group of retroperitoneal lymph nodes containing yellow granules. Hemorrhagic areas are present in some of the nodes.

FIG. 2. Section from a retroperitoneal lymph node showing large fatty agglomerates marginated by mononuclear foam cells and multinucleated giant cells.

FIG. 3. Section from a peritoneal lymph node showing markedly dilated lymph sinuses containing large mononuclear phagocytic foam cells and multinucleated giant cells.



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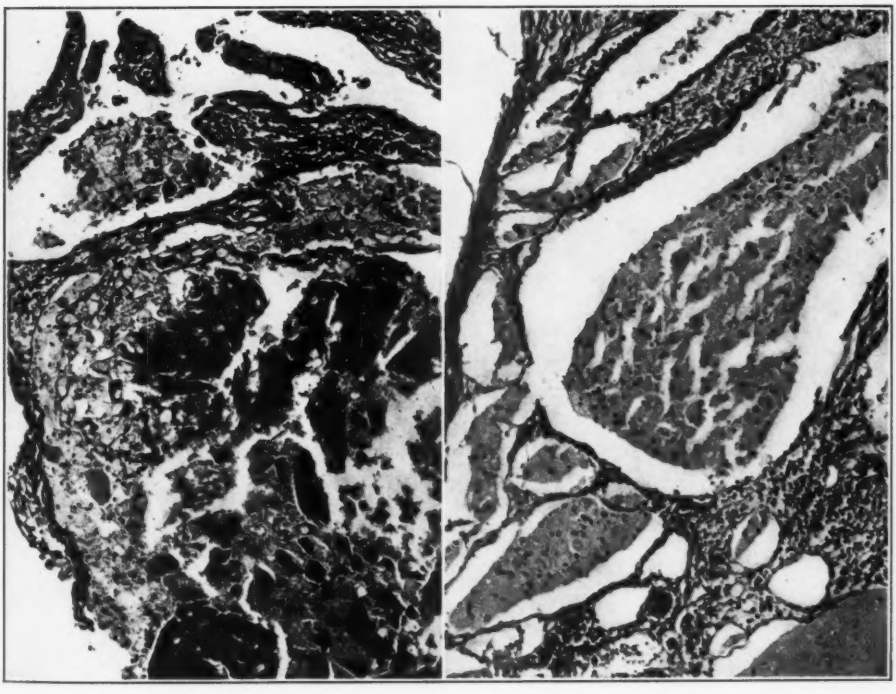

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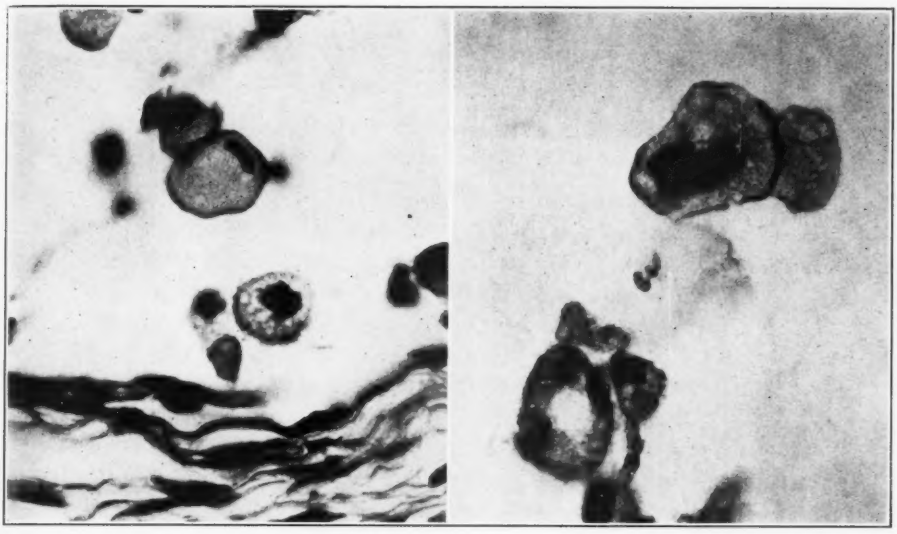
Plate 81

Figs. 4, 6 and 7. Mononuclear phagocytic cells with vacuolated cytoplasm and foam cells.

Fig. 5. Large multinucleated giant cells with vacuolated cytoplasm.



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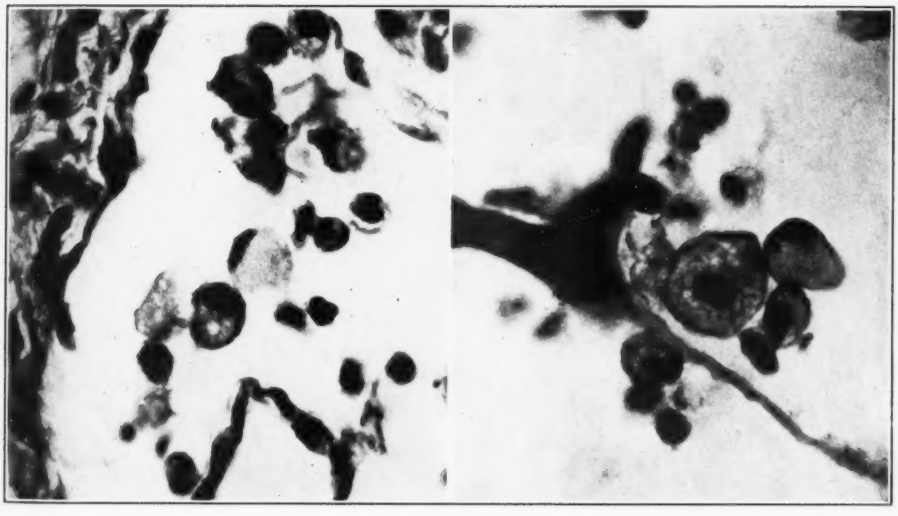

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THE EFFECT OF CERTAIN FACTORS ON THE RESULTS OF SILVER IMPREGNATION FOR RETICULUM FIBERS*

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This paper deals with the results of further experiments carried out in order to determine the effect of various factors on the results of silver impregnation of reticulum. The results of the first set of experiments were published in 1937,[1] together with the description of a simple method for the impregnation of reticulum in paraffin sections.

The Effect of Different Fixatives: As the results of most silver impregnation methods, either for reticulum or for other tissue elements, are known to depend on the type of fixation, and as in most instances the use of some specified fixative such as formalin, cobalt nitrate-formalin, alcohol, chloral hydrate, and others is recommended, it seemed to be of interest to ascertain the actual effect of different fixatives on the results of the stain. The following fixatives were used: alcohol, Carnoy's fluid, formalin-alcohol (1:5), formalin (1:10), Bouin's, Orth's, Zenker's and Stieve's fluids, and neutral Zenker-formalin (9:1). The material used was various human and animal (dog, guinea pig) organs, mainly kidneys, liver, spleen, heart muscle and pancreas. The impregnation of paraffin sections was done according to the method previously published.

It should be stated first of all that for practical purposes any one of the fixatives tried gives entirely satisfactory results. The reticulum fibers show up quite distinctly and selectively, and in about equal numbers after any of the fixatives. The difference is to be found in the staining of the nuclei and of the cytoplasm much more than in the reticulum stain itself. The best results consisting of deep black staining reticulum fibers, sharply stained gray nuclei and almost completely unstained cytoplasm, are obtained after fixation in Carnoy's fluid. A somewhat darker and often purplish cytoplasmic stain with a varying nuclear stain is

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obtained after Zenker's fixative. The results obtained with Zenker-formalin are quite similar. After fixation in Bouin's fluid the nuclei are entirely unstained while the cytoplasm is often darker than desirable. With all the other fixatives the results do not differ materially from those obtainable after formalin fixation. There can be no doubt that from an aesthetic point of view the results after fixation in Carnoy's fluid are much superior to those secured with other fixatives. I have found, however, that results almost identical with those obtained by fixation in Carnoy's fluid can be obtained after any fixative with a slight modification of the technic which I call "exhaustive oxidation" of the sections. This term means thorough oxidation of all tissue substances oxidizable with potassium permanganate. The potassium permanganate solution should be acidified with about 0.5 per cent sulphuric acid, and after decolorization with potassium metabisulphite the process should be repeated as often as there is the slightest brown staining of the sections by potassium permanganate (as a rule 2 to 3 times). Pale pink staining of the sections indicates the complete exhaustion of all substances capable of reducing potassium permanganate. After this type of oxidation subsequent mordanting and silver impregnation will bring out surprisingly sharp black and white contrasts. Sections must be affixed to the slide securely because they are easily loosened by oxidation and have a great tendency to float off.

Length of Time of Fixation: This does not seem to make much difference, at least not with formalin fixation, as tissues fixed for from 12 hours up to several months gave almost identical results.

Decalcification: Decalcification in either nitric or sulphosalicylic acid does not call for any modification in the technic as it has no effect whatsoever on the results of the impregnation.

Thickness of Sections: The thinner the sections, the better the result. With thick sections the picture is often blurred, the background being stained an unpleasant greenish or brownish gray, while the reticulum fibers are exceedingly pale. The limit of safety is about 8 μ, though occasionally excellent results may be obtained with sections as thick as 16 μ. Celloidin sections, either stained loose or affixed to slides, often show the same poor staining, especially if the celloidin has not been removed. As the importance of diffusion processes in silver impregnation is well known (an

especially interesting example being given in the Warthin and Starry technic [2]), it is by no means surprising that the thickness of the sections may not only quantitatively but also qualitatively influence the results.

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A DIFFERENTIAL STAIN FOR CELL TYPES IN THE PANCREATIC ISLETS *

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Most, if not all, of the accepted staining methods for the differentiation of cell types in the islets of Langerhans are more or less capricious and unreliable. The neutral gentian violet stain gives beautiful results in guinea pig material, but it is much less dependable in other animal species. The Mallory-Heidenhain azan method can be applied to almost all animal species, but gives a clear definition of the *alpha* granules only, while the *beta* granules are poorly demonstrated. The other methods (copper and iron hematoxylin, acid fuchsin-methyl green) are even less satisfactory than the stains mentioned.

The routine hematoxylin-eosin stain shows in well fixed tissues a definite difference between *alpha* and *beta* cells owing to the relative oxyphilia of the former and the basophilia of the latter. As a result of my attempts to increase this contrast I have found a fairly simple modification of the hematoxylin-eosin stain which brings out the two cell types more distinctly than any of the other stains used for the same purpose. It has the additional advantage of giving uniform results in all animal species examined (guinea pig, rat, mouse, cat, dog, rabbit, *Macacus rhesus* monkey, beef) as well as on human material. Its results were excellent in certain human cases in which all other stains failed. The two cell types were clearly demonstrated in both the normal and the diabetic pancreas sometimes taken as late as 4 hours after death. This method is also suitable for study of the degranulation processes in the pancreas in experimental animals. It can also be recommended for the pituitary. In the pancreas the *beta* granules stain a deep slate blue color, while the *alpha* granules stain red. The D cells of Bloom are not demonstrated. In the pituitary the basophils are blue, the oxyphils red, and the chromophobes are almost unstained.

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METHOD

Fixation: The thickness of pieces of tissue should not exceed 2 mm. in order to ensure rapid and complete penetration by the fixative. Aqueous fixatives, such as formalin, Bouin's, Zenker's, and Stieve's solution, or Zenker-formalin, may be used. The best fixative proved to be a modified Bouin's solution in which half the amount of acetic acid is replaced by sulphosalicylic acid (formalin 1 part, saturated picric acid solution 4 to 5 parts, acetic and sulphosalicylic acid 2.5 per cent each). For use dilute this fixative with equal parts of distilled water. The staining of tissues fixed in any fluid can sometimes be greatly improved by refixing the sections before staining in the undiluted solution for 12–24 hours. The removal of mercury salts from tissues is done by the routine iodine method.

Embedding: Embed tissues in paraffin.

Oxidation: Deparaffinized (and refixed, if necessary) sections are treated for 1 minute with a solution containing about 0.3 per cent each of potassium permanganate and of sulphuric acid. The sections are rinsed in water and then decolorized with a 1 to 5 per cent solution of potassium metabisulphite. After decolorization the sections are thoroughly washed in water. Without oxidation the *beta* granules will not take the stain. For sections of pituitary, oxidation, though it will enhance the color contrast, is not strictly necessary.

Staining: Stain in a well ripened solution of chromium hematoxylin for from 15 minutes to 1 hour under microscopic control. The *beta* granules should be a deep blue and the cytoplasm of the *alpha* cells should be unstained. In the pituitary the basophils should be a deep blue and the oxyphils unstained. Any mucoid material is stained a deep blue. The staining solution is made up as follows:

Mix equal parts of a 1 per cent aqueous solution of hematoxylin and of a 5 per cent solution of chromium alum (potassium chromium sulphate). The brownish mixture is ripened by the addition of about 3.5 cc. of a 5 per cent potassium dichromate solution plus a few drops of sulphuric acid to each 100 cc. of the mixture. The process of ripening will take 1 or 2 days. The solution is ready for use as soon as it is a deep blue-black with a

purplish tinge. It should be filtered before use as a precipitate will form repeatedly on its surface. For some time the staining power and selectivity of the solution increase, but as the solution becomes older the staining time has to be prolonged. Solutions that do not stain deeply enough in 35 to 40 minutes are better discarded as they often do not stain with sufficient precision.

After staining in this chromium hematoxylin solution rinse the sections in water and transfer to alcohol containing 1 per cent of hydrochloric acid. In this acid alcohol solution the color of the section becomes a clearer blue. Rinse again and counterstain rather heavily with any of the routine red or orange acid dyes. Phloxine and ponceau de xylidine have been found to be especially suitable. The latter is used in a 0.5 per cent solution with 1 per cent of acetic acid. Rinse in water and differentiate in a 5 per cent solution of phosphotungstic acid until the acid dye is completely removed from the connective tissue. Only the strongly oxyphilic structures, such as erythrocytes, muscle fibers, oxyphils and *alpha* cells, remain stained. Carry sections through graded alcohols, clear in xylol and mount in balsam.

DESCRIPTION OF PLATE

PLATE 82

Tissues were fixed in modified Bouin's solution and stained according to the method recommended. Phloxine was used as a counterstain.

An orange-yellow filter was used for photographing the sections. The blue stained tissue elements are dark and the pink stained elements pale. All microphotographs were taken at a magnification of × 380.

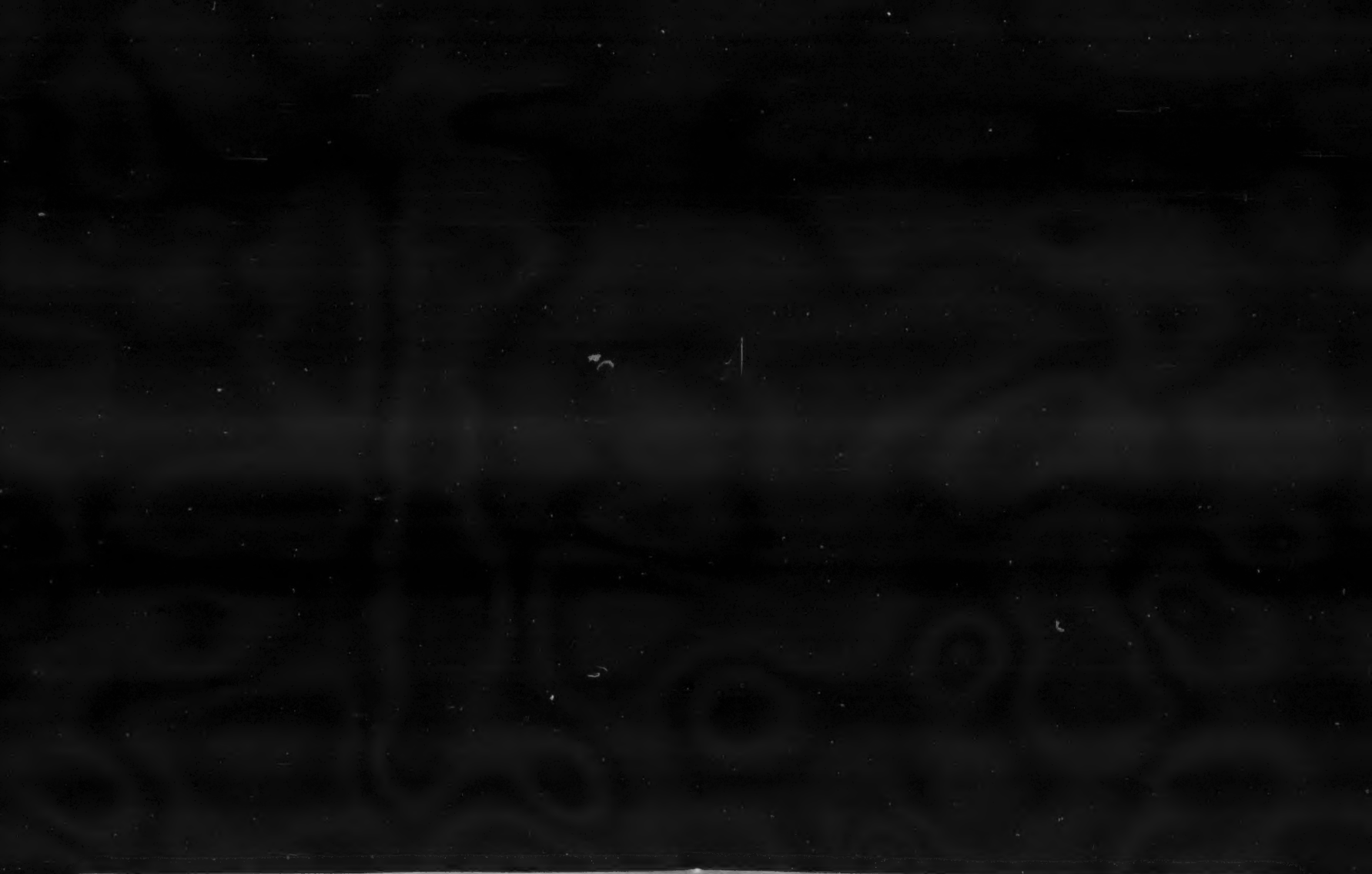
FIG. 1. Pancreas from a normal guinea pig. A = pale *alpha* cells; B = dark *beta* cells.

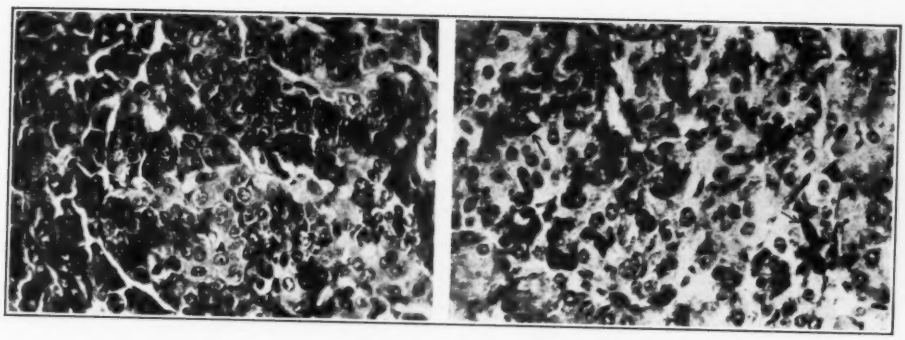
FIG. 2. An islet in the pancreas of a guinea pig. The blood sugar level was kept at 200 mg. per 100 cc. for 12 hours preceding death. Profound degeneration of the *beta* cells, of which only a few show very deep blue granules in a cap-shaped area of the cytoplasm, is to be seen.

FIG. 3. Normal human islet. A = pale *alpha* cells; B = dark *beta* cells.

FIG. 4. Human pancreas from a case of severe diabetes. A = pale *alpha* cells; B = dark *beta* cells; C = masses of hyalin.

FIG. 5. Anterior lobe of a human pituitary gland. A = chromophobes with unstained cytoplasm; B = dark basophils; C = pale oxyphils.





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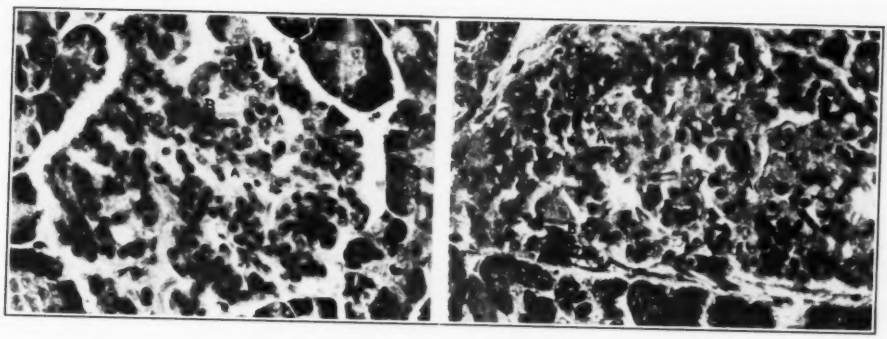

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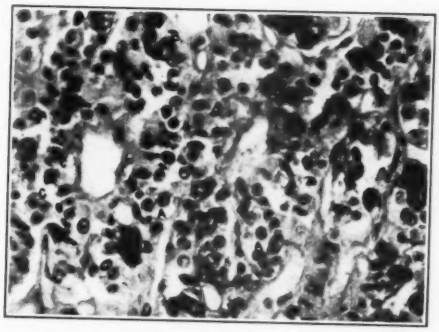

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Gomori Stain for Cell Types in Pancreatic Islets